Systemic Review on the potentials of Enzymes in the Bioremediation of crude oil polluted soils

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ABSTRACT

Petroleum is the major source of energy for various industries and daily life. Releasing petroleum into the environment whether accidentally or due to human activities is a main cause of soil pollution. Soil contaminated with petroleum has a serious hazard to human health and causes environmental problems as well. Petroleum pollutants, mainly hydrocarbon, are classified as priority pollutants. The application of microorganisms or microbial processes to remove or degrade contaminants from soil is called bioremediation. Bioremediation is an innovative technique or process that uses microorganisms or their enzymes or plant enzymes to detoxify contaminant in the soil and other environments. It degrades or reduces hazardous organic pollutants to innocuous compounds such as CO_2, CH_4, H_2O and biomass without adversely affecting environment. This microbiological decontamination is claimed to be an efficient, economic and versatile alternative to physicochemical treatment. As bioremediation can be effective only where environmental conditions permit microbial growth and activity, the limitation of bacterial growth is under the influence of pH, temperature, oxygen, soil structure, moisture and appropriate level of nutrients, poor bioavailability of contaminants, and presence of other toxic compounds. Microbial enzymes commonly used in bioremediation include oxidoreductases, laccases, peroxidases, lipases, cellulases, and proteases. This article present a review about bioremediation of petroleum-contaminated soil and enzymes (usually microbial origin) used in bioremediation.

1.0 INTRODUCTION

One of the major problems encountered as we approach a new century is environmental pollution. Environmental pollution cannot be accepted as inevitable for technological society. Soil contaminated by petroleum products is a pervasive problem in oil producing countries. The cleanup of these contaminated soils is a severe challenge. Regulations have mandated the contaminant limits for various petroleum products. Consideration of the current technology for removal of pollutants clearly shows that physical and chemical methods are often uneconomical. Hssssssowever, biological treatment technology may offer a solution to this problem (Chi-Yuan and Krishnamurthy 1995). The world depends on oil. Vast amount is used, transported, processed and stored around the world. In 2003, the total world consumption of petroleum was over 13.1 billion liters per day. The United States Energy Information Administration projects (as of 2006) world consumption of oil to increase to 98.3 million barrels per day ($15.63x10^6$ m^3 day^{-1}) in 2015 and 118 million barrels day^{-1} ($18.8x10^6$ m^3 day^{-1}) in 2030 (EIA, 2006). With such a high consumption, oil spills are inevitable.

The most notable oil spills at sea involve large tankers, such as Exxon Valdez, which spilled thousands of tones oil (Paine *et al*., 1996; Albaiges *et al*., 2006). These oil spills can cause severe damage to sea and shoreline organisms (Whitfield, 2003). Most responsible for the contamination are service stations, garages, scrap yards, waste treatment plants, sawmills and wood impregnation plants. Thereafter, several studies have been examined the fate of petroleum in various ecosystem (Boehm *et al*., 1995; Whittaker *et al*., 1999). The development of petroleum industry into new frontiers, the apparent inevitable spillages that occur during routine operations and records of acute

accidents during transportation has called for more studies into oil pollution problems (Timmis *et al*., 1998), which has been recognized as the most significant contamination problem (Snape *et al*., 2001). Oil is a complex mixture of hydrocarbons and other organic compounds, including some organometallic constituents (Butler and Mason, 1997). It contains hundreds or thousands of aliphatic, branched and aromatic hydrocarbons (Prince, 1993; Wang *et al*., 1998), most of which are toxic to living organisms (ATSDR, 1995).

In the past, wastes were traditionally disposed by digging a hole and filling it with waste material. This mode of waste disposal was difficult to sustain owing to lack of new place every time to dump. New technologies for waste disposal that use high-temperature incineration and chemical decomposition (e.g. base-catalyzed dechlorination, UV oxidation) have evolved. Although they can be very effective at reducing wide a range of contaminants but at the same time have several drawbacks. These methods are complex, uneconomical, and lack public acceptance. The associated deficiencies in these methods have focused efforts towards harnessing modern day bioremediation process as a suitable alternative.

Bioremediation is a microorganism mediated transformation or degradation of contaminants into nonhazardous or less-hazardous substances. The employability of various organisms like bacteria, fungi, algae, and plants for efficient bioremediation of pollutants has been reported (Vidal, 2001; Leung, 2004). Borah and Yadav (2016) reported that bioremediation provides the most cost-effective and eco-friendly measurements for the remediation of petroleum contaminated soil and water to bring back its native environment. Remediation refers to removing, degrading or transforming contaminants to harmless or less harmful substances. It includes methods that reduce mobility and migration of the contaminants, preventing their spreading to uncontaminated areas; toxicity of the contaminants remains unaltered, but the risk they pose to the environment is reduced

(US.DOD, 1994). The main molecules in crude oils and refine products are biodegradable, and they will eventually leave the environment as they are consumed by microbes. Bioremediation aims to stimulate the rate of this process (Prince, 1993). Bioremediation is an innovative technique or process that uses microorganisms or their enzymes or plant enzymes to detoxify contaminant in the soil and other environments. It degrades or reduces hazardous organic pollutants to innocuous compounds such as CO_2, CH_4, H_2O and biomass without adversely affecting environment (Ron and Rosenberg, 2014).

1.2 Factors affecting bioremediation of petroleum hydrocarbon pollutants

The process of bioremediation mainly depends on microorganisms which enzymatically attack the pollutants and convert them to innocuous products. As bioremediation can be effective only where environmental conditions permit microbial growth and activity, its application often involves the manipulation of environmental parameters to allow microbial growth and degradation to proceed at a faster rate. The limitation of bacterial growth is under the influence of pH, temperature, oxygen, soil structure, moisture and appropriate level of nutrients, poor bioavailability of contaminants, and presence of other toxic compounds. Although microorganisms can exist in extreme environment, most of them prefer optimal condition a situation that is difficult to achieve outside the laboratory (Vidal, 2001; Bernhard-Reversat and Schwartz 1997; Dua *et al.*, 2002; Dana and Bauder, 2011). The process of bioremediation is a very slow process. Only certain species of bacteria and fungi have proven their ability as potent pollutant degraders. Many strains are known to be effective as bioremediation agents but only under laboratory conditions. Most bioremediation systems operate under aerobic conditions, but anaerobic environments may also permit microbial degradation of recalcitrant molecules. Both bacteria and fungi rely on the participation of different

intracellular and extracellular enzymes respectively for the remediation of recalcitrant and lignin and organo-pollutants (Vidal, 2001; Hammel, 1997).

1.3 Microbial Enzymes in Bioremediation

1.3.1 Microbial Oxidoreductases

The detoxification of toxic organic compounds by various bacteria and fungi and higher plants through oxidative coupling is mediated with oxidoreductases (Gianfreda *et al.*, 1999; Bollag and Dec 1998). Microbes extract energy via energy yielding biochemical reactions mediated by these enzymes to cleave chemical bonds and to assist the transfer of electrons from a reduced organic substrate (donor) to another chemical compound (acceptor). During such oxidation-reduction reactions, the contaminants are finally oxidized to harmless compounds (ITRC 2002). The oxidoreductases participate in the humification of various phenolic substances that are produced from the decomposition of lignin in a soil environment. In the same way, oxidoreductases can also detoxify toxic xenobiotics, such as phenolic compounds, through polymerization, copolymerization with other substrates, or binding to humic substances (Park *et al.*, 2006). Microbial enzymes have been exploited in the decolorization and degradation of azo dyes (Park *et al.*, 2006). Many bacteria reduce the radioactive metals from an oxidized soluble form to a reduced insoluble form. During the process of energy production, bacterium takes up electrons from organic compounds and use radioactive metal as the final electron acceptor. Some of bacterial species reduce the radioactive metals indirectly with the help of an intermediate electron donor. Finally precipitant can be seen as the result of redox reactions within the metal-reducing bacteria (Leung, 2004). Chlorinated phenolic compounds are among the most abundant recalcitrant wastes found in the effluents generated by the paper and pulp industry. These compounds are produced

upon the partial degradation of lignin during pulp bleaching process. Many fungal species are considered to be suitable for the removal of chlorinated phenolic compounds from the contaminated environments. The activity of fungi is mainly due to the action of extracellular oxidoreductase enzymes, like laccase, manganese peroxidase, and lignin peroxidase, which are released from fungal mycelium into their nearby environment. Being filamentous, fungi can reach the soil pollutants more effectively than bacteria (Rubilar *et al.,* 2008). Water polluted with phenolic compounds can be decontaminated by plants with the help of enzymes exuded by their roots. The plant families of Fabaceae, Gramineae, and Solanaceae are found to release oxidoreductases which take part in the oxidative degradation of certain soil constituents. Phytoremediation of organic contaminants has been generally focused on three classes of compounds: chlorinated solvents, explosives, and petroleum hydrocarbons (Dur´an and Esposito, 2000; Newman *et al.*, 1998).

1.3.2 Microbial Laccases

Laccases (p-diphenol:dioxygen oxidoreductase) constitute a family of multicopper oxidases produced by certain plants, fungi, insects, and bacteria, that catalyze the oxidation of a wide range of reduced phenolic and aromatic substrates with concomitant reduction of molecular oxygen to water (Gianfreda *et al*., 1999; Mai *et al.,* 2000). Laccases are known to occur in multiple isoenzyme forms each of which is encoded by a separate gene (Giardina *et al.,* 1995), and in, some cases, the genes have been expressed differently depending upon the nature of the inducer (Rezende *et al.,* 2005). Many microorganisms produce intra and extracellular laccases capable of catalyzing the oxidation of ortho and paradiphenols, aminophenols, polyphenols, polyamines, lignins, and aryl diamines as well as some inorganic ions (Mai *et al*., 2000; Ullah *et al*., 2000; Couto and Toca Herreram, 2006). Laccases not only oxidize phenolic and methoxyphenolic acids, but also

decarboxylate them and attack their methoxy groups (demethylation). These enzymes are involved in the depolymerization of lignin, which results in a variety of phenols. In addition, these compounds are utilized as nutrients for microorganisms or repolymerized to humic materials by laccase (Kim *et al.*, 2002). Among the biological agents, laccases represent an interesting group of ubiquitous, oxidoreductase enzymes that show promise of offering great potential for biotechnological and bioremediation applications (Gianfreda *et al.,* 1999). The substrate specificity and affinity of laccase can vary with changes in pH. Laccase can be inhibited by various reagents such as halides (excluding iodide), azide, cyanide, and hydroxide (Xu, 1996). Different laccases appear to have differing tolerance toward inhibition by halides, indicating differential halide accessibility. Laccase production is sensitive to the nitrogen concentration in fungi. High nitrogen levels are usually required to obtain greater amounts of laccase. Recombinant laccase can be produced by either homologous or heterologous means (Gianfreda *et al.,* 1999).

1.3.3 Microbial Peroxidases

Peroxidases (donor: hydrogen peroxide oxidoreductases) are ubiquitous enzymes that catalyze the oxidation of lignin and other phenolic compounds at the expense of hydrogen peroxide (H2O2) in the presence of a mediator. These peroxidases can be haem and nonhaem proteins. In mammals, they are involved in biological processes such as immune system or hormone regulation. In plants, they are involved in auxin metabolism, lignin and suberin formation, cross-linking of cell wall components, defense against pathogens, or cell elongation (Hiner *et al.*, 2002; Koua *et al.*, 2009). The hemeperoxidases have been classified into two distinct groups as found only in animals and found in plants, fungi, and prokaryotes. The second group peroxidases have been subdivided into three classes on the basis of sequence comparison. Class I is intracellular enzymes including yeast cytochrome c peroxidase, ascorbate peroxidase (APX) from plants, and bacterial gene-duplicated

catalase peroxidases. Class II consists of the secretory fungal peroxidases such as lignin peroxidase (LiP) and manganese peroxidase (Mnp) from Phanerochaete chrysosporium, and Coprinus cinereus peroxidase or Arthromyces ramosus peroxidase (ARP). The main role of class II peroxidases appears to be the degradation of lignin in wood. Class III contains the secretory plant peroxidases such as those from horseradish (HRP), barley or soybean. These peroxidases seem to be biosynthetic enzymes involved in processes such as plant cell wall formation and lignifications (Hiner *et al.,* 2002; Koua *et al.,* 2009). Nonhaem peroxidases are not evolutionarily linked and form five independent families. They are thiol peroxidase, alkylhydroperoxidase, nonhaem haloperoxidase, manganese catalase and NADH peroxidase. Among all these thiol peroxidase is the largest and having two subfamilies such as glutathione peroxidases and peroxy redoxins (Koua *et al.,* 2009).

1.3.4 Microbial Lipases

Lipase degrades lipids derived from a large variety of microorganisms, animals and plants. Recent works have shown that lipase is closely related with the organic pollutants present in the soil. Lipase activity was responsible for the drastic reduction total hydrocarbon from contaminated soil. Research undertaken in this area is likely to progress the knowledge in the bioremediation of oils spill (Margesin *et al.,* 1999; Riffaldi *et al.,* 2006). Lipases have been extracted from bacteria, plant, actinomycetes, and animal cell. Among these microbial lipases are more versatile because of their potent application in industries. These enzymes can catalyze various reactions such as hydrolysis, interesterification, esterification, alcoholysis and aminolysis (Sharma *et al.,* 2011). Lipases are ubiquitous enzymes which catalyze the hydrolysis of triacylglycerols to glycerol and free-fatty acids. Lipolytic reactions occur at the lipid-water interface, where lipolytic substrates usually form equilibrium between monomeric, micellar, and emulsified states. Lipases have been classified into

two types on the basis of criteria such as (a) enhancement in enzyme activity as soon as the triglycerides form an emulsion and (b) lipases with a loop of protein (lid) covering on the active site (Joseph *et al.,* 2006). Triglyceride is the main component of natural oil or fat. This can hydrolyze consecutively to diacylglycerol, monoacylglycerol, glycerol, and fatty acids. Glycerol and fatty acids are widely used as raw materials, for instance, monoacylglycerol is used as an emulsifying agent in the food, cosmetic, and pharmaceutical industries. The study made on trioleinhydrolysis from Candidarugosalipase inthebiphasic oil-water system as proven to be effective. The lipase adsorbs on to the oil-water interface in the bulk of the water phase. The lipase then breaks the ester bonds of triolein to produce consecutively diolein, monoolein, and glycerol. During the catalysis oleic acid is formed at each consecutive reaction stage. The glycerol formed is hydrophilic and thus dissolves into the water phase (Hermansyah *et al.,* 2007). Lipase activity was found to be the most useful indicator parameter for testing hydrocarbon degradation in soil (Margesin *et al.,* 1999; Riffaldi *et al.,* 2006). Lipase is of much interest in the production of regiospecific compounds which are employed in pharmaceutical industry. Along with its diagnostic usage in bioremediation, lipase has many potential applications in food, chemical, detergent manufacturing, cosmetic, and paper making industries, but its production cost has restricted its industrial use (Sharma *et al.,* 2011; Joseph *et al.,* 2006).

1.3.5 Microbial Cellulases

Cellulases now promise the potential of converting waste cellulosic material into foods to meet burgeoning population and have been the subject of intense research (Bennet *et al.,* 2002). Some organisms produce cell bound, cell envelope associated, and some extra cellular cellulases. Extracellular cellulases, hemicellulases, and pectinases have been shown to be constitutively expressed at very low levels by some bacteria and fungi (Rixon, 1992; Adriano-Anaya *et al.,*

2005). Cellulases are usually a mixture of several enzymes. At least: three major groups of cellulases are involved in the hydrolysis process (1) endoglucanase (EG, endo- 1,4-Dglucanohydrolase) which attacks regions of low crystallinity in the cellulose fiber, creating free chain ends; (2) exoglucanase or cellobiohydrolase (CBH, 1,4-b-D-glucan cellobiohydrolase) which degrade the cellulose molecule further by removing cellobiose units from the free chain ends; (3) βglucosidase which hydrolyzes cellobiose to glucose units. Along with major enzymes, some ancillary enzymes are also present. During the enzymatic hydrolysis, cellulose is degraded by the cellulases to reducing sugars that can be fermented by yeasts or bacteria to ethanol (Sun and Cheng, 2002). Cellulase enzymes are capable of degrading crystalline cellulose to glucose. Cellulases have been used in the manufacture of detergents since early 1990s.

1.3.6 Microbial Proteases.

Proteases hydrolyze the breakdown of proteinaceous substance which enter atmosphere due to shedding and moulting of appendages, death of animals, and also as by product of some industries like poultry, fishery, and leather. Proteases belong to group of enzymes that hydrolyze peptide bonds in aqueous environment and synthesize them in nonaqueous environment. Proteases have wide range of applications in food, leather, detergent, and pharmaceutical industry (Singh, 2003; Beena and Geevarghese, 2010). Proteases are divided as endopeptidases and exopeptidases based on the catalysis of peptide chain. Endopeptidases further grouped based on the position of active site such as serine endopeptidase, cysteine peptidase, aspartic endopeptidases, and metallopeptidases. The enzymes whose reaction mechanism is completely elucidated are grouped under. The exopeptidases act only near the terminal amino or carboxylic position of chain. The protease that acts on free amino, and carboxyl terminals are called as aminopeptidase and carboxypeptidase, respectively. The endopeptidase acts on the inner regions of peptide chain. The

presence of free amino and carboxyl terminal will have negative impact on enzyme activity (Beena and Geevarghese, 2010).

CONCLUSION

The quality of life on earth is linked inextricably to the overall quality of the environment. For centuries, we believe that atmospheric, terrestrial and aquatic systems were sufficient to absorb and breakdown wastage from population centre, industry and farming. However, today the resources in the world show greater or lesser degree due to our carelessness and negligence in using them. The problems associated with petroleum contaminated sites assume an increasing prominence in many countries. These pollution problems often result in huge disturbances of both the biotic and abiotic components of the ecosystem. The currently accepted disposal methods are incineration or burial in secure landfills can become prohibitively expensive when the amounts of contaminants are large. Microbial remediation of an oil contaminated site is accomplished with the help of a diverse group of microorganisms, particularly the indigenous bacteria present in soil. This process is known as bioremediation. It uses relatively low-cost, low technique, which generally have a high public acceptance and can often be carried out on site.

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