The review of literature pertaining to the present study entitled “Evaluation of Polyethylene Degrading Potential of Isolated Soil Bacteria” is presented under the following headings.

2.1 Plastics - Types and classification

2.2 Impact of plastics on environment

2.3 Bioremediation strategies

2.4 Microbial degradation of polyethylene

2.5 Role of ligninolytic enzymes in biodegradation

2.6 Role of Biosurfactants in Bioremediation

2.1 Plastics - Types and classification

Plastics are polymeric material that has the capability of being molded or shaped, usually by the application of heat and pressure. This property of plasticity, often found in combination with other special properties such as low density, low electrical conductivity, transparency, and toughness, allows plastics to be made into a great variety of products. Plastics are important materials of our society and the world demand and production of plastics are higher than ever (Arabiourrutia et al., 2012).

The commonly used plastic products include tough and lightweight beverage bottles made of polyethylene terephthalate (PET), flexible garden hoses made of polyvinyl chloride (PVC), insulating food containers made of foamed polystyrene, and shatterproof windows made of polymethyl methacrylate. Plastic debris is composed of several different polymers, and their unique chemical ingredients may make some types of plastic more hazardous than others when their chemical constituents are bioavailable to organisms. For example, polyvinyl chloride (PVC), polycarbonate, polyurethane and polystyrene (PS) are composed of hazardous monomers (e.g., vinyl chloride, bisphenol-A and styrene) and/or contain hazardous additives (e.g. PBDEs, phthalates and lead) (Rochman et al., 2014).
Plastics consist of carbon, hydrogen, silicon, oxygen, chloride and nitrogen. Oil, coal and natural gas are used for extraction of the basic materials of plastics. The plastics of various forms such as nylon, polycarbonate, polyethylene terephthalate, polyethylene, polypropylene, polystyrene, poly tetra fluoroethylene, polyurethane, polyvinyl chloride are being continuously used in our day to day life (Sangale et al., 2012).

Polymers that are classified as plastics can be divided into two major categories: thermoplastics and thermosets. Thermoplastics such as polyethylene and polystyrene are capable of being molded and remolded repeatedly. Thermosets, on the other hand, cannot be reprocessed upon reheating. During their initial processing, thermosetting resins undergo a chemical reaction that results in an infusible, insoluble network. Annual worldwide manufacture of thermoplastics has increased from less than two million tonnes in 1950 to between 230 and 245 million tonnes during the last decade (Harrison et al., 2014).

Worldwide production of plastics is increasing by approximately 5% every year, because plastics exhibit advantages such as clarity, hardness, processability and lightness together with price competiveness (Yoon et al., 2012).

Plastics can be classified into four categories considering their biodegradability and raw materials. Polyethylene (PE), polypropylene (PP), and poly (ethylene terephthalate) (PET), which have contributed to the development of today's human society, are typical oil-based non-biodegradable plastics. Whereas most of the oil-based plastics are recognized as non-biodegradable, poly (e-capro-lactone) (PCL), poly (butylene succinate/adipate) (PBS/A), and poly (butylene adipate- co -terephthalate) (PBA/T) are biodegradable (Tokiwa and Pranamuda, 2002).

Among the various types of plastic polymers, the most popular and convenient plastic polymers include low-density polyethylene (LDPE), high-density polyethylene (HDPE), polypropylene (PP), polyvinyl chloride (PVC), polystyrene (PS), nylon, polyethylene terephthalate (PET) and polyurethanes (Sangeethadevi et al., 2015).

Polythene is very useful in our day to day life to meet our desired needs. It can be used for wrapping the goods, food material, medicine and scientific instruments. Due to its good quality its use is increasing day by day. Among the synthetic plastics waste produced, polythene shares about 64%. Polythene is made from the cheap petrochemical stocks extracted from oil or gas through efficient catalytic polymerization
Polyethylene (Figure 1) belongs to an important class of materials and is used in many technological sectors. Low-density polyethylene, especially, is often used in the automotive, medical, aerospace and electronics fields (Sanchis et al., 2008).

LDPE is prepared from gaseous ethylene under very high pressures (up to about 350 megapascals, or 50,000 pounds per square inch) and high temperatures (up to about 350 °C [660 °F]) in the presence of oxide initiators. These processes yield a polymer structure with both long and short branches. Because the branches prevent the polyethylene molecules from packing closely together in hard, stiff, crystalline arrangements, LDPE is a very flexible material. Its melting point is approximately 110 °C (230 °F). Synthetic polymers like low-density polyethylene are mostly inert towards microorganisms. In the initially produced form, they undergo very slow biodegradation in both stabilized and un-stabilized forms (Raut et al., 2015).

HDPE is manufactured at low temperatures and pressures. The lack of branches in its structure allows the polymer chains to pack closely together, resulting in a dense, highly crystalline material of high strength and moderate stiffness. With a melting point more than 20 °C (36 °F) higher than LDPE, it can withstand repeated exposure to 120 °C (250 °F) so that it can be sterilized. It is well-known that high and low density polyethylene (HDPE and LDPE) and polypropylene (PP) are the main plastics in municipal solid waste around the world. Several reports have been published on different ways for chemical or petrochemical feedstock recycling of PE and PP, because it results in hydrocarbon products like fuel after pyrolysis (Yan et al., 2015).
Linear low-density polyethylene (LLDPE) is structurally similar to LDPE. It is made by copolymerizing ethylene with 1-butene and smaller amounts of 1-hexene and 1-octene, the resultant structure has a linear backbone, but it has short, uniform branches that, like the longer branches of LDPE, prevent the polymer chains from packing closely together. Overall, LLDPE has similar properties to LDPE and competes for the same markets. Linear low-density polyethylene is a suitable polymer for biomedical applications due to its good physiochemical properties (Popelka et al., 2014).
During the past three decades, plastic materials are increasingly used in food, clothing, shelter, transportation, construction, medical and recreation industries. These drastic rises in the use of plastic materials have not been accompanied by the corresponding development of procedures for the safe disposal or degradation of these materials. Polyethylene represents up to 64% of the synthetic plastics produced, and they are mainly used for the manufacturing of plastic bags, bottles, disposable containers, which are discarded within a short time (Balasubramaniam et al., 2010).

2.2 Impact of plastics on environment

The annual global production of the organic polymer has rapidly increased from 1.7 to 280 million tonnes within the last 60 years resulting in the accumulation of plastic litter in virtually all habitats (Lechner et al., 2014). Plastic wastes represent a serious concern for the environment because of its recalcitrance to microbial attack. Degradation of waste plastics through microorganisms represents one of the alternatives to deal with such problems. Microorganisms such as bacteria and fungi are involved in the degradation of both natural and synthetic plastics (Gu, 2003).

Plastic pollution, originating from sea- and land-based sources, migrates into subtropical gyres where it forms accumulation zones of microplastic particles distinct from surrounding waters relatively free of plastic pollution (Eriksen et al., 2013). Ingestion of marine debris by wildlife, and that of plastics by seabirds in particular, has been widely documented. The northern fulmar Fulmarus glacialis was among the earliest seabird species reported to ingest marine plastic debris (Franeker and Law, 2015).

Plastic pollution is the dominant type of anthropogenic debris ubiquitous throughout the marine environment. Plastic pollution enters the marine environment via rivers, beaches, maritime activities, and illegal dumping at sea (Ryan et al., 2009). Under the effects of UV degradation and hydrolysis, plastic loses its elasticity, and powered by wind and waves, gradually breaks into smaller particles (Cole et al., 2011). In other studies of marine debris, primarily from coastal assessments, 60–80% of marine debris is petroleum-based plastic (Derraik, 2002).

All of the marine organism groups are at an eminent risk of interacting with microplastics according to the available literature. Dozens of works on other relevant issues (i.e., polymer decay at sea, new sampling and laboratory methods, emerging sources, externalities) were also analysed and discussed (do Sul and Costa, 2014).
Recycling of waste plastics can be a good alternative solution. However, collection and cleansing of waste plastics are an expensive process, and the physical properties decay seriously during the reprocessing procedure (Yoon et al., 2012). The pollution profiles of volatile organic compounds (VOCs) emitted from different recycling workshops processing different types of plastic solid waste (PSW) and their health risks were investigated. At the high temperature (more than 400 °C), PSW could be converted into valuable substances, which can be used as either fuels or feedstock in the petrochemical industry. Although the melting temperature in the mechanical recycling is around 150–300 °C which is much lower than the pyrolysis, various volatile organic compounds (VOCs) might still be produced and released into the atmospheric environment at this temperature due to the aging, long thermal exposure, intrinsic sensitivity, and the interactions between the additives and polymers (He et al., 2015).

The synthetic plastics are thus, dumped into landfills or are incinerated. Incineration burns off the plastic waste completely, but at the same time causes heavy toxic fume generation (Al-Salem et al., 2009; Crowley et al., 2003). Recycling is a very environmentally-attractive solution, but a very small part of the plastics can be recycled where the remaining goes to the burial sites (Bhatia et al., 2014).

These polymers feature ester bonds, which can be degraded by certain enzymes that are secreted by microorganisms, and are thus categorized as oil-based biodegradable plastics. Biodegradable plastics and bio-based plastics are often confused with each other as eco-friendly plastics, although they are not identical in terms of the original concept. Biodegradable plastics have been developed from the viewpoint of biodegradability, whereas for bio-based plastics, biomass is used as the raw material instead of oil (Iwata, 2015).

Under environmental conditions, larger plastic items degrade to so-called microplastics (MP), fragments typically smaller than 5 mm in diameter. MP are of special concern since their bioaccumulation potential increases with decreasing size (Wagner et al., 2014).

MP may be ingested by various organisms ranging from plankton and fish to birds and even mammals, and accumulate throughout the aquatic food web (Wright et al., 2013). In addition, plastics contain a multitude of chemical additives (Dekiff et al., 2014) and adsorb organic contaminants from the surrounding media (Bakir et al., 2014).
Since these compounds can transfer to organisms upon ingestion, MP act as vectors for other organic pollutants (Zarfl and Matthies, 2010).

Pollution with plastic debris and microplastic fragments has been recognized as a major problem in fresh and marine water systems (Derraik, 2002; Andrady, 2011). Negative effects may relate to entanglement in plastic wires or nets, or to ingestion, which has been reported for benthic invertebrates, birds, fish, mammals and turtles (Koelmans et al., 2014).

2.3 Bioremediation strategies

Bioremediation using microorganisms and plants to detoxify or remove pollutants owing to their diverse metabolic capabilities is an evolving method for the removal and degradation of many environmental pollutants including the products of petroleum industry. Microorganisms play key roles in biotransformation of complex contaminant mixtures during soil bioremediation processes (Gómez et al., 2007; Gadd, 2010).

There are many methods for the removal of these pollutants from soils. They involve both physicochemical and biological approaches. Although the physicochemical methods are more effective than biological methods, they are expensive and require high energy demand and consumption of many chemical reagents. This is a reason why use of microorganisms capable of degrading toxic compounds known as bioremediation has become an attractive technology (Hamdi et al., 2007).

Bioremediation is a low-cost technology, whose efficacy is often enhanced with preliminary mild physicalchemical remediation methods. The selected microbial strains *M. populi* VP2 and *A. sydowii* VP4, isolated from a highly contaminated industrial soil, were effective in the bioaugmentation treatment of aqueous contaminated extracts obtained from the same polluted soil (Sannino et al., 2016). Natural attenuation uses indigenous microorganisms to degrade contaminants and has the advantage of being natural, involving no external modification of the environment (Mills et al., 2003).

One of the in situ bioremediation strategies is bioaugmentation, which improves the biodegradative capacities of contaminated sites by introduction of single strains or consortia of microorganisms with desired catalytic capabilities. Moreover, genetically engineered microorganisms (GEMs) exhibiting enhanced degradative capabilities encompassing a wide range of aromatic hydrocarbons have also potential for soil bioaugmentation (Mrozik and Piotrowska-Seget, 2010).
Bioaugmentation, based on inoculating microorganisms with the required degradation capability, might be a way of enhancing the biodegradability of toxic contaminants. Bioaugmentation with competent degrading strains of bacteria can stimulate the rate and the extent of biodegradation in appropriate environments (Lang et al., 2016).

There are two main approaches to oil spill bioremediation: Bioaugmentation (addition of oil-degrading bacteria) and biostimulation (stimulating the growth of indigenous oil degraders by the addition of nutrients or other growth-promoting co-substrates) (Das and Chandran, 2010).

Studies have shown that plants have the ability to detoxify some xenobiotics in soil by direct uptake of the contaminants, followed by subsequent transformation, transport and product accumulation (Macek et al., 2008). The physicochemical and biological characteristics, e.g., soil organic matter content, bulk density, porosity, permeability, soil respiration and material transfer process, can be altered by the high hydrophobicity of hydrocarbons (Liang et al., 2012).

2.4 Microbial degradation of polyethylene

An increased environmental damage was noticed recently due to the amount of plastic wastes persisting many years after disposal. A partial solution is to replace conventional, petrochemical commodity plastics with biodegradable substitutes (Lovino et al., 2008). Microorganisms exist in this environment could develop potential to biodegrade the recalcitrant pollutants when they are exposed to polluted environment without addition of chemicals in the system, the acquired potential at the contaminated site by bacteria are reported in literatures (Kotresha and Vidyasagar, 2008).

Biodegradation is the ability of microorganism to influence abiotic degradation through physical, chemical or enzymatic action (Albertsson et al., 1987). Biodegradation of polyethylene is known to occur by two mechanisms: Hydro-biodegradation and oxo-biodegradation. These two mechanisms agree with the modification due to the two additives, starch and pro – oxidant, used in the synthesis of biodegradable polyethylene. Starch blend polyethylene has a continuous starch phase that makes the material hydrophilic and therefore, catalyzed by amylase enzymes. Microorganisms can easily access, attack and remove this part. Thus the hydrophilic polyethylene matrix continues to be hydro-biodegraded. In case of pro- oxidant additive, biodegradation occurs
### Table 1. List of microorganisms associated with polymer degradation

<table>
<thead>
<tr>
<th>Type of polymer</th>
<th>Microorganisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyurethane</td>
<td><em>Comamonas acidovorans</em> TB-35, <em>Curvularina senegalensis</em>, <em>Fusarium solani</em>, <em>Aureobasidium pullulans</em>, <em>Cladosporium sp.</em>, <em>Trichoderma DIA-T</em> spp., <em>Trichoderma sp.</em>, <em>Pestalotiopsis microspora</em></td>
</tr>
<tr>
<td>Poly(3-hydroxybutyrate)</td>
<td><em>Pseudomonas lemoignei</em>, <em>Alcaligenes faecalis</em>, <em>Schegelella thermodepolymerans</em>, <em>Aspergillus fumigatus</em>, <em>Penicillium spp.</em>, <em>Penicillium funiculosum</em></td>
</tr>
<tr>
<td>Poly(3-hydroxybutyrate-co-3-hydroxvalerate)</td>
<td><em>Clostridium botulinum</em>, <em>C. acetobutylicum</em>, <em>Streptomyces sp. SNG9</em></td>
</tr>
<tr>
<td>Polycaprolactone</td>
<td><em>Bacillus brevis</em>, <em>Clostridium botulinum</em>, <em>C. acetobutylicum</em>, <em>Amycolatopsis sp.</em>, <em>Fusarium solani</em>, <em>Aspergillus flavus</em></td>
</tr>
<tr>
<td>Polylactic acid</td>
<td><em>Penicillium roquefort</em>, <em>Amycolatopsis sp.</em>, <em>Bacillus brevis</em>, <em>Rhizopus delemar</em></td>
</tr>
</tbody>
</table>

(Pathak and Navneet, 2017)
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following photo degradation and chemical degradation. If the pro-oxidant is a metal combination, after transition, metal catalyzed thermal peroxidation and biodegradation of low molecular weight oxidation products occur sequentially (Bonhomme *et al.*, 2003).

Biodegradability has become a useful characteristic for plastics. Conversely, the introduction of biodegradable plastics has generated a need for methods to evaluate the biodegradation of these polymers in landfills and solid waste treatment systems such as composting or anaerobic digestion treatment plants (Orhan and Büyükgüngör, 2000).

Biodegradable materials have gained academic and industrial importance particularly for the protection of the environment from ever increasing plastics waste. Partially biodegradable materials prepared by blending natural biodegradable and non-biodegradable synthetic polymers can reduce the volume of plastics waste by their non-complete environmental biodegradation (Morro *et al.*, 2016).

Most of the researchers recommend pretreatment for efficient microbial adherence. Such treatments result in the formation of carbonyl, carboxyl and ester functional groups that decreases the hydrophobicity. This is the process in which various byproducts such as CO₂, water, methane and other inorganic substances are formed depending on whether the process is aerobic or anaerobic. (Shah *et al.*, 2013)

Microbial attack is started where the carboxylic acid group is generated through Norrish Type I and II mechanism during oxidation. The mechanism consists of generation of terminal carboxylic acid group, which then undergoes beta oxidation. The manner and rate are dependent on the mechanism of degradation and on the acceleration of the process, respectively (Raut *et al.*, 2015).

The rate of biodegradation has been reported to be affected by irradiation with UV light and chemicals, by surface area, by antioxidant, by additives and also by the molecular weight of the plastic (Kyaw *et al.*, 2012). To facilitate biodegradation of these polymers a preliminary step of photo-oxidation or thermo-oxidation has routinely been employed. Abiotic degradation includes the physical and/or chemical processes that exert intra molecular modifications in the polymer (Sivan, 2011).

There are numerous studies aiming at increasing biodegradability of polypropylene by creating polar groups, such as carbonyl, carboxy, and ester groups, through addition of pro-oxidants or by UV irradiation. The degree of PP biodegradation by microbes is
examined by either observing the change in the morphology, measuring the mass reduction or monitoring decrease in the molecular weight and tensile strength (Jeon and Kim, 2016).

Broader spectrum of isolated taxons showed that the microorganisms capable of adhering to preoxidized polyethylene surface and metabolizing some of its oxidation products are not rare in forest soils and because the identified genera are relatively common, an assumption could be made, that similar results can be expected in other types of soils with rich microbial communities. If the prooxidant technology ensures continuation of the polyethylene chain breakdown process after PE waste disposal, one can expect in soil, such oxidation products to be biodegraded (Koutny et al., 2009).

Because of their metabolic diversity and environmental adaptability the bacteria became a better resource for remediation. A better understanding of the enzymatic system involved in polyethylene degradation could be useful in the development of remediation approaches for plastic wastes which could eliminate plastic pollution concerns (Yang et al., 2014).

The microbes release the extracellular enzymes such as lignin peroxidase and manganese peroxidase to degrade the polythene but the detailed characterization of these enzymes in relation to polythene degradation is still to be carried out (Sangale et al., 2012).

Polyethylene films containing pro-oxidant additives in their formulations have been introduced in outdoor plastics markets as new promising biodegradable materials. Primary applications of these degradable materials are mainly in the mulching and packaging film applications as well as other products of limited lifetimes e.g. carry bags. (Abrusci et al., 2013). Transition metals especially iron, cobalt and manganese have the ability to decompose the hydroperoxides formed during oxidative degradation of polymers and therefore, they are sometime used in commercial photodegradable formulations. The oxidation state of the metal (II or III) in the case of co-stearate, did not affect their ability to initiate and accelerate polyethylene degradation (Roy et al., 2008).

The rate of abiotic degradation of polyethylene films containing a manganese pro-degradant has been studied in various environments at 60° and 70°C. It is proposed that ammonia and/or hydrogen peroxide generated by microorganisms in the compost can be responsible for the deactivating effect, as aqueous solutions of these compounds

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significantly retard the rate of degradation (Jakubowicz *et al.*, 2006). Biodegradation of polyurethane has been reported in several bacteria or fungi isolated from soil. Bioremediation of potentially hazardous pulp and paper mill effluent by a laccase producing *paenibacillus* sp. strain LD-1 isolated from contaminated soil (Raj *et al.*, 2014).

Bacteria often switch from a free living lifestyle to a surface adapted, multicellular organization known as biofilm. Indeed, in most habitats—both natural and artificial—the majority of microbial populations form biofilms on solid surfaces. Bacteria in biofilms become highly differentiated from those in the planktonic state and often exhibit a developmental sequence, forming complex, multicellular structures (microcolonies), which become surrounded by a network of water channels (Sivan *et al.*, 2006).

The effective bioremediation techniques for refractory and hazardous waste need preliminary investigations of novel degraders and clarifications of the main mechanism for their microbial degradation (Peng *et al.*, 2014). *Trichoderma harzianum* was isolated from dumpsites for the use in the biodegradation of polyethylene (Sowmya *et al.*, 2014).

There is a growing interest in synthetic polymer biodegradation using effective microorganisms. Developments of microbial communities attached to the synthetic wastes have been found to be powerful degrading agents in nature. Studies on fungal mediated degradation of polyethylene has previously been reported using *Mucor rouxii* and *Aspergillus flavus* (El-Shafei *et al.*, 1998), *Phanerochaete chrysosporium* (Liyoshi *et al.*, 1998), *Penicillium simplicissimum* YK (Yamada-Onodera *et al.*, 2001) and *Aspergillus niger* ITCC no. 6052 (Mathur *et al.*, 2011).

Studies on polyethylene biodegradation (Albertsson, 1980; Albertsson *et al.*, 1987), including the biotic environment (Shah *et al.*, 2008), have been reported. However, few studies have been conducted on non-amended polyethylene (Balasubramanian *et al.*, 2010; Hadad *et al.*, 2005).

In most studies, fungi were considered for the degradation of LDPE due to their ability to form hydrophobic proteins that can attach to the polymer surface, their generation of degrading enzymes that are well matched to the insoluble LDPE, the faster growth of fungal biomass in soil compared to bacteria, and the growth extension and penetration into other locations through the distribution of hyphae. Also, fungi survive environments with low nutrient, pH and moisture availability (Raut *et al.*, 2015).
There have been many reports of the survival of pathogenic Staphylococcal strains in close proximity of plastic material, it is evident that the organism not only survived in an atmosphere where shredded LDPE was its only assimilable carbon source, but also proliferated. This was a clear indication that the organisms had some mechanism for degrading the plastic (Figure 2) and utilize its metabolites for its own growth (Chatterjee et al., 2010).

**Figure 2. Mechanism of biodegradation** (Raut et al., 2015)
The studies using enrichment techniques gave rise to the isolation of several unique soil bacteria able to grow on low-density polyethylene as a sole carbon source, reducing up to 11% of its gravimetric weight in 30 days (Santo et al., 2012). Biodegradation of the polyethylene was obtained with pre-irradiated (to obtain partial photooxidation) or non-irradiated films. The maximal degrading activity was obtained with Rhodococcus ruber strain C208 (Gilan et al., 2004) and with the thermophilic bacterium Brevibacillus borstelensis strain 707 (Hadad et al., 2005).

Polymers such as poly (lactic acid), poly(butylene adipate-co terephthalate), poly(ε-caprolactone), and starch-based polymer blends or copolymers can degrade when exposed to bioactive environments such as soil and compost (Kasirajan and Ngouajio, 2012). A unique method of biodegradation of commercial polyethylene by using simultaneously a bio-surfactant produced by Bacillus licheniformis and Lysinibacillus bacterium in various combinations was investigated. Polyethylene is biodegraded via conversion of carbonyl groups into unsaturated hydrocarbon by both bio-surfactant and Lysinibacillus bacterium (Mukergee et al., 2016).

Culture-based seawater microcosm studies have also demonstrated microbial attachment to polyethylene terephthalate and polyethylene plastic bags. The colonization of plastic by microorganisms can additionally be considered as biofouling, with such biofilm formation suggested to contribute towards changes in the buoyant density of polymers, leading to transport of plastic from the oceanic surface waters into the deeper water column and into sediments. Understanding the ecology and biodiversity of the plastisphere, and unravelling these systems will require the characterization of plastic fragments isolated across diverse environments, as well as exposure experiments that can effectively mimic the complex journeys of plastic pollutants within aquatic ecosystems (Harrison et al., 2014).

The green polyethylene was developed from renewable raw materials to help to reduce the emissions of greenhouse gases. P. ostreatus is a lignocellulolytic fungus that can use lignin, cellulose and hemicellulose as carbon and energy sources. This fungus has been used in the degradation of agroindustrial residues, the bioremediation of pollutants and pulp bleaching. The ability of lignocellulolytic fungi to degrade a large range of compounds is related to the high efficiency of their enzymatic system.
The exposure of GP to sunlight and its subsequent incubation in the presence of *P. ostreatus* can decrease the half-life of GP and facilitate the mineralization of these polymers (da Luz *et al.*, 2015).

Recently, several three-dimensional structures of enzymes that catalyze the degradation of biodegradable plastics, for example, polyhydroxybutyrate depolymerase and cellulases were revealed (Iwata *et al.*, 2015).

A mesophilic bacterium capable of Low-Molecular-Weight Polyethylene (LMWPE) biodegradation was isolated from a beach soil having been contaminated extensively with crude oil. The isolated strain was rod-shaped gram negative bacterium and was identified as *Pseudomonas* sp. E4 (Yoon *et al.*, 2012).

### 2.5 Role of ligninolytic enzymes in biodegradation

The ligninolytic enzymes are a ubiquitous group of enzymes found in different types of organisms as plants, bacteria, insects, and fungi. The microorganisms possess an enzymatic system to effectively degrade lignin. This system contains three principal enzymes laccase, manganese peroxidase (MnP), and lignin peroxidase (LiP). These microorganisms have been used to delignify different substrates such paper, animal feed, and biofuels (Placido and Capareda, 2015).

The ligninolytic enzyme system of white rot fungi (comprising lignin peroxidase (LiP, E.C. 1.11.1.14), manganese peroxidase (MnP, E.C. 1.11.1.13) and laccase (E.C. 1.10.3.2) along with H₂O₂-producing oxidases) has low substrate specificity, non sterio selectivity and strong oxidative abilities and Versatile peroxidase (VP) and many accessory enzymes such as glyoxal oxidase, aryl alcohol oxidase, oxalate producing oxalate decarboxylase and P-450 mono-oxygenase have also been isolated from culture filtrates of many white rot fungal strains (Asgher *et al.*, 2014).

Laccase belongs to copper-containing oxidases and catalyzes the four electron reduction of molecular oxygen to water. It is a glycoprotein that contains four copper atoms per monomer distributed in three redox sites termed as type1 (T1) or blue Cu, type2 (T2) or normal Cu and type3 (T3) or coupled binuclear Cu. Type2 and type3 coppers form a trinuclear cluster. Since laccase recycles on molecular oxygen as an electron acceptor and does not require any other co-substrate, it is the most promising enzyme of oxidoreductases group for industrial applications (Yadav and Yadav, 2015).
The bacterial ligninolytic potential is still largely unexplored and many novel ligninolytic enzymes may await discovery. These bacterial enzymes may be superior to their fungal counterparts with regard to specificity, thermostability and mediator dependency. They may also have specific advantages for the depolymerization of the modified lignin residues typically encountered in waste streams from the pulping or second generation bio based industrial chemicals (Bandounas et al., 2011).

The role of bacterial blue multicopper proteins in different cellular processes, like sporulation (Bacillus), resistance to copper (Escherichia coli) and LAC activity have been reported only recently (Palanisami et al., 2010).

Recently it has become clear that bacteria are relatively rich in another type of peroxidase, the so-called dye-decolorizing peroxidases (DyPs, EC 1.11.1.19). DyPs represent a newly discovered family of heme-containing peroxidases, which has recently received attention due their ability to degrade lignin and other compounds. Already in 1988 a bacterial ‘lignin peroxidase’ was described from Streptomyces viridosporus (de Gonzalo et al., 2016).

A number of bacteria capable of degrading lignin have been reported. Of those bacteria, several Bacillus sp. strains have been reported as lignin degrading bacteria. Bacillus sp. (CSA105) was isolated from sediment core from the pulp and paper mill industries and purified ligninolytic enzyme from the cell extract. In addition, several kraft lignin-degrading Bacillus sp. have been isolated. These results indicate that Bacillus sp. may be an important microorganism and may play a key role in lignin biodegradation (Chang et al., 2014).

However, information about bacterial peroxidase is very limited due to a lack of identified microorganisms and enzymes exhibiting ligninolytic potential. Until now, Streptomyces viridosporus, Pseudomonas putida, and Rodococcus jostii have been reported as bacterial strains being involved in lignin degradation (Ahmad et al., 2011; Yang et al., 2012).

In nature, lignin peroxidase can oxidize both phenolic and non-phenolic lignin-related compounds, resulting in the cleavage of the Cα–Cβ bond and the aryl Cα bond, aromatic ring opening, phenolic oxidation, and demethoxylation (Christian et al., 2005).

Laccase has been used for numerous biotechnological applications, such as drug analysis to distinguish morphine from codeine, wine clarification, and most
importantly, in the degradation of several recalcitrant pollutants such as trichlorophenol, alkenes, bisphenol A and fluorene as well as herbicides and other persistent and toxic molecules, in addition to decolorization of synthetic azo dyes (Mayer and Staples, 2002).

Laccases and certain fungal class II peroxidases from white-rot basidiomycetes are considered in degradation of persistent organic pollutants. Bioaugmentation, introduction of for example white-rots for enzyme production into a polluted soil, and biostimulation of suitable resident organisms by nutritional manipulations are strategies in degradation of pollutants in soil. Bioaugmentation has been successfully implemented on small scale for soils in biobeds and for specific materials such as olive mill wastes (Kues, 2015).

In accordance with the stimulation of laccase expression, copper enhanced polyethylene biodegradation revealed by FTIR spectra indicated an increase of more than 40% in the carbonyl index. Thus, laccase may play a role in oxidation of the hydrocarbon backbone of polyethylene. One of the most important indicators of polyethylene biodegradation is molecular weight reduction (Sivan, 2011).

2.6 Role of Biosurfactants in Bioremediation

Many microorganisms produce extracellular or membrane associated surface-active compounds (biosurfactants). Most microbial surfactants are complex molecules, comprising different structures that include lipopeptides, glycolipids, polysaccharide protein complexes, fatty acids and phospholipids. In the past few decades, biosurfactants have gained attention because of their advantages such as biodegradability, low toxicity, ecological acceptability and ability to be produced from renewable and cheaper substrates (Desai and Banat, 1997; Nitschke et al., 2004).

The range of industrial applications of biosurfactants includes enhanced oil recovery, crude oil drilling, lubricants, bioremediation of pollutants, health care and food processing (Banat et al., 2000). Biosurfactants offer several advantages over their chemical counterparts – such as their ecological acceptance, biodegradability, low toxicity, potentially high activities, effectiveness and stability at extremes of temperature, pH and salinity and possibility of production from renewable substrates. *Pseudomonas* is known to produce low molecular mass rhamnolipid biosurfactants with excellent surfactant properties (George and Jayachandran, 2013).
Unlike chemically synthesized surfactants, which are classified according to the nature of their polar grouping, biosurfactants are categorized mainly by their chemical composition and their microbial origin. Structurally they are amphiphilic molecules that comprise both hydrophobic and hydrophilic moieties, being the apolar component usually a carbon chain, whereas the polar part, more variable, can be ionic (anionic or cationic) or non-ionic (Pereira et al., 2013).

Most known biosurfactants are glycolipids. They are carbohydrates in combination with long-chain aliphatic acids or hydroxyaliphatic acids. Among the glycolipids, the best known are rhamnolipids, trehalolipids, and sophorolipids (Desai and Banat, 1997). Several types of low molecular weight lipopeptide biosurfactant are produced by members of Bacillus species and their extensive application includes agriculture, cosmetic, food petroleum and pharmaceutical industries (Das and Chandran, 2010).

Many types of biosurfactants have been synthesized from bacteria belonging to a wide variety of genera, such as Acinetobacter, Arthrobacter, Pseudomonas, Halomonas, Bacillus, Rhodococcus and Enterobacter. The lipopeptides produced by numerous Bacillus sp. are classified into three families depending on their amino acids sequence: surfactins, iturins and fengycins (Bezza and Chirwa, 2015)

The IR and UV spectra indicated the possible lipopeptide nature of the crude biosurfactant. Similar spectral characters for lipopeptide surfactants from Bacillus clausii (Hazra et al., 2015), Bacillus pumilus (Morikawa and Imanaka, 1992) and Bacillus licheniformis (Yakimov et al., 1995) have been reported.

The capability of biosurfactants to enhance organic contaminants availability and biodegradation rates was reported by many authors (Chrzanowski et al., 2012; Ławniczak et al., 2013). Studies revealed that biosurfactants can enhance hydrocarbon bioremediation by two mechanisms: The first include the interaction with the poorly soluble contaminants and improving their transfer into the aqueous phase. While the other involves their role in enhancing the biodegradation efficiency (Ayed et al., 2015).

Currently, the use of agroindustrial by products for the production of biosurfactants is one of the much adopted fermentation methods as they serve as cheaper and economical substrates for higher yields and also helps in waste disposal. The use of low cost substrates is an essential factor for the overall economic recovery as they account for 50% of the total production cost (Makkar and Cameotra, 1999;
Rodrigues et al., 2006). Several studies reported the use of agro-industrial wastes such as molasses, whey milk, distillery waste, olive oil mill effluent, soap stock, cassava waste and potato substrates (Makkar and Cameotra, 1999; Henkel et al., 2014; Gudiña et al., 2015) and vegetable oils as carbon substrates (Rahman et al., 2002; Wu et al., 2008; Oliveira et al., 2009).