# MICROBIAL ENZYMES IN THE RECYCLING OF WASTES

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## SUMMARY

The increasing volume of different types of wastes from various sources is an important environmental problem due to the ever growing migration and successive urbanization. Enzymes are biological catalysts from plant, animals or microorganisms with numerous potential applications. Microbial enzymes have been exploited in the recycling and management of wastes through enzymatic degradation and remediation resulting into less toxic useful products. Microbial enzymes are classified based on their mechanism of action as oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases with oxidoreductases and hydrolases being the most utilized in waste treatment and recycling. Microbial oxidoreductases are involved in catalyzing oxidation-reduction reaction in harmful biodegradable materials to non-toxic products. The oxidoreductases employed in waste degradation include the oxygenases (mono and di-oxygenases), laccases and peroxidases. Microbial hydrolases catalyze the breakdown of waste biomass especially from food, agricultutral, chemical and biomedical industries by addition of water molecules to the waste materials. Some microbial enzymes with hydrolytic properties includes cellulase and hemicellulase, protease, lipase, amylase, lactase, xylanase and pullulanase. Compared to conventional chemical methods, recycling of wastes using microbial enzymes have great significance in bioremediation as they are specific, fast, relatively cheap, applied across a wide variety of contaminants, greatly reduce the waste and at the same time produce useful products. However, microbial enzymes are not devoid of limitations such as selection of the most suitable microbial enzyme for recycling and the ability of these enzymes to retain their active nature under normal conditions of operation for a prolonged period of time. Exploitation of molecular studies will in the near future provide a clearer picture on mechanisms of enzyme action either singly or in consortium with other enzymes during biodegradation and recycling of wastes to more valuable products.

**Keywords**: Biodegradation, bioremediation, enzymes, microorganisms, recycling, waste.

#  INTRODUCTION

The overall quality of the environment is inextricably linked and highly dependent on the quality of life on planet Earth (IIheme *et al*. 2017). The growing human population generates wastes of various kinds on a daily basis as a result of a wide range of different activities (Ebikapade and Jim 2016). This leads to a continuous accumulation in the volume and variety of wastes and thus poses a great threat to the earths’ flora and fauna (Vergara and Tchobanoglous 2012). The pollution of soil and water by industrial chemicals, petroleum hydrocarbons, polythene, plastics, metallic materials and glass wastes are serious problems of the modern world. Due to their extensive use, they are found as environmental contaminants in numerous aquatic and terrestrial ecosystems.

Recycling is described as the reprocessing of used materials into new products. This prevents or decreases utilization of raw materials and consumption of energy (Magram 2011). The purpose of recycling is to convert waste products that could be land filled or out of stream waste and use as feed-stocks or raw materials for new or useful products (Dyson and Chang 2005). Waste recycling poses a major environmental and economic challenge worldwide, it takes place out of sight, hence attract less public concern and least priority for authorities (Dyson and Chang 2005).

Naturally, wastes are spontaneously recycled by plants and microorganisms especially bacteria and fungi to maintain a healthy ecosystem (Figure 1). Microbial enzymes are biological catalyst mostly proteins produced by microorganisms especially bacteria, yeast and moulds and have been exploited greatly in medicine, industries and biotechnology (Periasamy *et al*. 2013). Compared to plant and animal enzymes, microbial enzymes are more recognized and preferred owing to their relatively high activity and stability coupled with the ease of production and recovery in large quantities (Shahid *et al*. 2016).

Researchers have identified enzymatic treatment of waste as an effective method of waste management compared to the conventional methods because of their selective and specific activity; they are not inhibited by most toxic substances; they have low retention time; they function over a wide range of concentration; they are less expensive and produced in large quantities; and they provide a safe and economic alternative (Aitken 1993; Karam and Nicell 1997).

# WASTES

Waste is any unwanted substance (solid, liquid or gas) or material regarded as useless and to be disposed of as being broken, contaminated or spoilt (Anifowose *et al*. 2011; Rajan*et al*. 2019; Ayilara *et al*. 2020). It is an unavoidable byproduct of human activities whose continuous generation results to loss of resources (Cheremisinoff 2003). Waste, now an important environmental problem, is as a result of increasing rate of development, urbanization and migration to the cities (Ayilara *et al*. 2020). The improper waste management is hazardous to the environment, humans and animals alike. Waste pollutes the air when burnt, release gases that deplete the ozone layer such as carbon dioxide, hydrogen and methane causing climate change (Bhat *et al*. 2018). When dumped in water, it affects aquatic lives, humans, soil organisms and plants by lowering the pH and depositing metals which increases the toxicity of water (Mani and Kumar, 2014; Sahay *et al*. 2019; Corral-Bobadilla *et al*. 2019; Holanda and Johnson 2020). It harbors vectors of diseases such as mosquitoes and put refuse workers at risk of injuries and infections (Alam & Ahmade 2013).

## Classifications of Wastes

Based on the state of matter, waste materials are classified as solid, liquid and gaseous wastes. On the basis of biodegradability, they are categorized as undegradable, partially degradable and completely degradable. Completely degradable (biodegradable) solid wastes are wastes that undergo decomposition by microorganisms into their diverse components (Alam and Ahmade 2013). Wastes from food, manure and from crop production can be decomposed completely (Lorenz *et al*. 2013). Agricultural wastes from animal sources such as cow dung, poultry droppings etc. are also classified as biodegradable wastes (Bhat *et al*. 2018). The purpose of biodegradation is to reduce volume of waste deposited, reduce its harmful impact on human health and environment by producing useful products with economic impact (Holm-Nielsen *et al*. 2009).

The non-biodegradable wastes are materials that cannot undergo biological or microbial decomposition or breakdown and these includes wastes from mines, mineral materials, polythene bags, leathers, plastics, glass etc (Baltrėnas *et al*. 2005). Non-biodegradable waste can be grouped into recycled and un-recycled waste. The recycled wastes are sold to companies and converted into new products while the un-recycled wastes are waste materials that are transported to dump sites and incinerated (Alam and Ahmade 2013).

Solid waste materials are classified based on whether they can be incinerated which are combustible and non-combustible and also based on the danger they posse or are associated with i.e. hazardous or non-hazardous (Demirbas 2011). The hazardous solid wastes are a public health threat to human, animals and the environment and these hazards include toxic gases, infectious diseases and corrosive substance. Waste materials that do not pose potential hazardous or harmful threats are classified as non-hazardous (Buragohain *et al*. 2020).

## Sources of Wastes

Waste comes from different sources, in different forms and dumped in different ways (Ahmed, 2013). The release of waste into the environment affects quality of life and the impact on environment is unquantifiable (Ahmed 2013; Tulebayeva *et al*. 2020). The significant sources of waste generation include municipal, agricultural, industrial, biomedical and Electronic wastes (Amasuomo & Baird 2016).

### **Municipal Waste**

Municipal solid waste (MSW) also known as garbage are waste collected from households, schools, markets, malls, gardens, streets, litter containers (Buragohain *et al*. 2020; OECD 2021). The increasing amount of MSW generation as a result of industrialization, migration, urbanization and improper disposal of food waste poses a serious global challenge (Rajan *et al*. 2018). It is generated from different sources where human activities take place. Developing countries generate about 55-80% of house hold waste and 10-30% of commercial and market waste which consist of industries, streets, institutions and many others (Nabegu 2017). There are risks associated with the improper management of MSW which threats to public health and environmental safety from collection to reusable materials (WHO 2015). Kaza *et al* (2018) estimated that globally by the year 2050, the generation of MSW will raise to 3.40 billion tonnes. The health risk associated with waste are higher in low income countries as a result of unpleasant methods of waste disposal such as uncontrolled dumping sites and burning of solid waste (Ferronato and Torretta 2019) with lower risks in high income countries.

Controlled dumping sites, incineration, land-filling, composite, anaerobic digestion and recycle are some of the methods of waste treatment and disposal (Kaza *et al*., 2018; Vinti *et al*., 2021).

### **Industrial Waste**

Industrial wastes are wastes produces as a result of industrial activities such as production of oil and gas, coal combustion, mining, products manufacturing (Demirbas 2011). By-products generated from manufacturing processes such as from mills mining, factories are also regarded as industrial waste materials. This waste also includes radioactive wastes, metals, paints, chemicals, sand papers and paper products. Wastes from industrial sources are potentially toxic pollutants that necessitate thoroughly treatment before discharge into the environment (Maczulak 2010).

### **Biomedical Waste**

Biomedical wastes are waste generated from healthcare institutions such as radioactive materials, blood, sharp and non-sharp objects, pharmaceutical products and chemicals (Nwachukwu *et al*. 2013). About 85% of waste generated in health care are non-hazardous and the remaining 15% are hazardous that may cause infection, toxicity to the environment or poisonous (radioactive) (WHO 2018). WHO (2018) reported about 16million injection being administered per year globally resulting in the improper disposal of needle and syringe after use. Waste generated from the health care centers exposes patients, care givers and waste handlers to potential infection, injuries and toxic materials at the same time polluting the environment such waste include radioactive materials, pharmaceutical wastes, non-hazardous waste pathological waste and toxic waste (Nwachukwu *et al*. 2013). To adequately manage health care waste, separation, appropriate treatment and safe disposal is important to enable proper recycling and disposal (Nwachukwu *et al*. 2013). Incineration of these wastes may result to the release of toxic chemicals and particles causing pollution. Therefore, proper actions should be taken to make sure environmental safety and health management are put in place to prevent serious health and environmental impact such as accidental release of chemicals and biological hazards including drug resistant microorganisms (WHO 2018).

### **Agricultural Waste**

The role of agriculture cannot be over emphasized in human and economic development with the growing human population, technological advancement towards green revolution and expansion of soil for agriculture production resulting in increased waste generation which may constitute serious public health challenge through pollution (Adejumo *et al*. 2020). Agricultural waste are wastes generated from growing and processing of raw farm products resulting to by- products that may be beneficial but have less economical value and high cost of management. Agro waste consists of animal and food waste, harmful and toxic agricultural waste (pesticides, herbicides, insecticides). The intensity of agriculture in developing countries may contribute to the increased generation of agro waste globally with about 998 million tons of agro waste generated yearly (Agamuthu 2009; Obi *et al*. 2016). Agricultural wastes are not properly managed because very little is known about the potential risks and benefits associated with proper management (Adejumo *et al*. 2020).

Agricultural waste can be utilized through: the absence of oxygen (anaerobic) digestion, fertilizer application, absorbent in the removal of heavy metals, pyrolysis, animal feed and direct combustion. Management of agro waste required the need to consider waste as potential resources rather than undesirable to avoid water, air and land contamination. Improper management of these wastes may also result in breeding place of insect with ability to transmit disease, soil quality and degradation such as phosphorous loading, emission of gases and foul odor such as ammonia and methane (Obi *et al*. 2016)

### **Electronic waste (e-waste)**

Electronic waste (e-waste) describes the discarded electrical or electronic devices. E-waste is among the types of waste plaguing the world currently. Used electronics which are destined for reuse, refurbishment, salvage and recycling through material recovery, disposal, or abandonment are also considered e-waste. The informal processing of e-waste in developing nations can result in adverse effects on human health and lead to environmental pollution. Scrap components of electronics, such as Central Processing Units (CPU), contain potential harmful materials such as cadmium, lead, beryllium, and brominated flame retardants (Buragohain *et al*. 2020). The recycling and disposal of e-waste can have significant risks to health of workers and the communities in developed and developing countries (Sakar 2016).

# MECHANISM OF ENZYME DEGRADATION OF WASTES

There are six groups in which all known enzymes are classified based on their mechanism of action and they include oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases. Out of the six classes, the oxidoreductases and hydrolases are the most employed in recycling of wastes.

## Oxidoreductases

These are group of enzymes that aid from a donor to an acceptor the transfer of oxygen, hydrogen, electrons or protons. Oxidoreductaces from microbial sources such as bacteria and fungi detoxify organic pollutants using oxidation-reduction reaction (Chandrakant and Shwetha 2011). Microorganisms extract energy from biochemical exothermic reactions through the breakdown of chemical bonds by these oxidoreductaces. These enzymes also utilize the energy for electron transfer from a donor organic compound to another accepting chemical compound. The donor is reduced while the acceptor is oxidized. Safer substances are generated from the pollutants after the redox reaction process (Cirino & Arnold 2002).

## Transferases

Functional groups e.g. acyl, alkyl, formal, glycosyl, hydroxymethyl, methyl, sulfate and phosphate groups are transferred using a nucleophillic substitution reaction from donor to an acceptor by this class of enzyme (Pandeeti *et al*. 2019).

## Hydrolases

This class of enzyme mediates the breakdown of carbon to carbon, carbon to oxygen and carbon to nitrogen bonds using water molecules (Chandrakant and Shwetha, 2011). Toxicity of pollutants are decreased through microbial hydrolase disruption of main chemical bonds (Vasileva-Tonkova and Galabova 2003). Alcoholysis and condensation reactions are also mediated by this class of enzyme. Constant availability, ability to withstand addition of solvent and absence of need to select co-factors are some of the merit of this class of enzyme (Schmidt, 2006). In biomedical sciences, chemicals, food and feed industries, hydrolases have been exploited greatly for their wide potential applications (Sanchez-Porro *et al*. 2003).

## Lyases

This enzyme class is responsible for catalyzing addition and elimination reactions. They cleave the bonds between carbon atoms (C–C) and carbon and other atoms such as oxygen (C–O), nitrogen (C–N) e.t.c by elimination. Lyases breakdown double bonds in chemical pollutants and subsequently, mediates the insertion of other chemical groups athwart the cleaved double bond (Pandeeti *et al*. 2019).

## Isomerases

The structural rearrangement of molecules i.e isomerization is facilitated by microbial isomerases.

## Ligases (Synthetases)

 Also known as synthetase, are enzymes with catalyzing ability of joining two large molecules together resulting into a new chemical bond. It is also generally associated with small chemical groups and linking of compounds. This class of enzymes establishes carbon-oxygen, phosphoric-ester, carbon-sulfur, carbon-nitrogen, and nitrogen-metal bonds, carbon-carbon (Lehninger, *et al*. 2004; Manjunuth *et al*. 2018).

Although, in many waste recycling techniques, two or more enzyme mechanisms is usually utilized.

# MICROBIAL ENZYMES IN WASTE DEGRADATION/RECYCLING

Microbial enzymes are explored continuously in the degradation, remediation and recycling of different wastes (Buragohain *et al*. 2020).

## Microbial Oxidoreductases

Oxidoreductases are a class of enzyme that catalyzes oxidation-reduction reactions. They are involved in the biodegradation of harmful waste compounds such as radioactive metals, halogenated compounds, phenolic and other related aromatic and aliphatic hydrocarbons (Vidali 2001; Park *et al*. 2006). Oxidoreductases from microorganisms have been employed for decolorization, degradation and remediation of azo and other related synthetic dyes (Leung, 2004; Husain, 2006). The microbial oxido-reductases most studied in waste bioremediation due to their high efficacy to degrade harmful substances in the environment include the oxygenases (mono and dioxygenases), laccases and peroxidase (Arora *et al*. 2010; Chandrakant and Shwetha 2011).

### **Microbial Oxygenases**

This family of oxido-reductases are responsible for the biodegradation of broad range of wastes materials by increasing their solubility, reactivity and breakdown of aromatic rings present in the wastes materials. The cleavage of the aromatic rings in the toxic wastes by oxygenases is achieved by introducing atoms of oxygen into the organic compounds (Fetzner 2003; Arora *et al*. 2009).Bacterial sources of oxygenases are the most researched in biodegradation and remediation of toxic waste materials (Chandrakant and Shwetha 2011).A large majority of oxygenases oxidize reduced toxic substrate using Flavin Adenine Dinucleotide (NAD), reduced nicotinamide adenine dinucleotide (NADH) or reduced nicotinamide adenine dinucleotide phosphate (NADPH) as co-substrates (Fetzner 2003).

The widespread use of insecticides, fungicides, herbicides and other chemicals containing high concentrations of halogens makes them a major environmental pollutant. Specific microbial oxygenases are being exploited in the breakdown of these toxic contaminants. Oxygenases have also being utilized in consortium with other multi-functional enzymes to catalyze the removal of these halogens in methane, ethane and ethylene containing compounds (Chandrakant and Shwetha 2011). In the process of pulp bleaching, chlorinated phenolic wastes from paper and pulp industries are generated in abundance from incomplete breakdown of lignin (Rubilar *et al*. 2008). Oxygenases from fungal sources are extracellular, and are released into nearby environments from the mycelium of fungi. Hence, due to this advantage, numerous suitable species of fungi are being exploited for biodegradation and bioremediation of environments contaminated with chlorinated phenolic compounds (Rubilar *et al*. 2008). Oxygenases are further grouped into monooxygenases and dioxygenases on the basis of number of oxygen atoms used for oxygenation.

#### **Microbial Mono-oxygenase**

This enzyme group consists of a vast super-family involved in catalysis of a variety of simple (e.g alkanes) to complex substrates (fatty acids and steroids) through oxidation reactions. Mono-oxygenases operates by integration into its substrates an atom from anoxygen molecule (Figure 2). Their relatively high stereo-selectivity on a broad variety of substrates makes them a useful tool in biodegradation and remediation processes. On the basis of the presence of co-factors, mono-oxygenases are divided into P450-dependentand flavin-dependent mono-oxygenases. The P450 mono-oxygenases usually contain iron and they are found in prokaryotic as well as eukaryotic organisms. The flavin-dependent monooxygenases on the other hand contain flavin as its prosthetic group and they generally require NADPH or NADP as coenzyme (Arora *et al*. 2010). Most mono-oxygenase require cofactor, although some members of this enzyme group can function properly without a cofactor, they require molecular oxygen for their action and exploit the substrate as a reducing agent (Cirino and Arnold 2002).

Mono-oxygenases have been employed in the biodegradation and biotransformation of a wide variety of aliphatic and aromatic contaminants through the removal of sulfur and halogens as well as addition of ammonia and hydroxyl groups. These properties have been explored in recent years for important application in recycling of recalcitrant wastes (Chandrakant and Shwetha 2011).

#### **Microbial Di-oxygenase**

This group utilizes a multi-component system to catalyze enantio-specification by introducing molecular oxygen into their substrate. In other words, di-oxygenases breakdown complex waste compounds by introduction of two atoms of oxygen into its substrates (Figure 3) to produce simpler products. They primarily oxidize aromatic compounds hence have been exploited in the remediation of pollutants in the environment. The dioxygenases usually have proteins used in electron transport which precedes their oxygenase components (Dua *et al*. 2002). Among other mechanism that the nature employ to breakdown of aromatic compounds in the environment is the utilization of catechol dioxygenases. The breakdown and subsequent biotransformation of aromatic compounds to produce simpler aliphatic compounds is catalyzed by catechol dioxygenase found to be manufactured by many soil bacteria. The extradiol degrading enzyme make use of Fe(II) and sometimes Mn (II) while the intradiol degrading enzyme the other hand exploit only Fe(III) (Chandrakant and Shwetha 2011).

### **Microbial Dehalogenase**

Microbial dehalogenase has significant applications in bioremediation of halogenated organic compounds. Dehalogenase enzyme degrades a wide range of halogenated compounds by breaking the alkyl-halide bonds (Wang *et al*. 2018) through three mechanisms: the hydrolytic, reductive, and oxygenolytic methods. Dehalogenation is performed by replacing atom of halogen with hydroxyl group from water molecules (Wang *et al*. 2018). *Bacillus* sp. with intrinsic ability to concurrently carry out debromination and mineralization of tribromophenol (TBP) has been reported (Zu *et al*. 2012). The bacteria utilize two pathways in the debromination step of which reductive bromination and methyl bromination is the major and minor pathways respectively, producing CO2 as the by-product of the mineralization (Zu *et al*. 2012). Other bacterial species such as *Pseudomonas umsongensis* YCIT1612 (Xue *et al*. 2018) other species of *Pseudomonas* (Liu *et al*., 1994), *Ancylobacter aquaticus* strain UV5, and *Rhizobium* sp. synthesize enzymes with the ability to transform a variety of halogenated pollutants (Kumar *et al*. 2016).

### **Microbial Laccases**

These are multi-copper-containing extracellular enzymes found in bacterial and fungal species and consist of mono, di, and tetrameric glycol-proteins. Microbial laccases are produced by different microorganisms. Laccase from *Streptomyces* sp. is well identified, characterized and most studied. Various species of *Streptomyces* have been found to produce laccase. They include *Streptomyces ipomoea*, *Streptomyces cyaneus, S. bikiniensis*, and *S. coelicolor*. Out of all the species *S. coelicolor* is the most broadly characterized (Guan *et al*. 2018). The presence of lignin and other phenolic compounds present in a wide variety of agricultural waste materials (e.g. banana peels, rice bran, maize husk, saw dust and other lignin rich materials) elicits the production of laccase by these organisms (Muthukumarasamy *et al*. 2015). Laccases are capable of oxidizing phenolic compounds, aromatic amine as well as derivatives of these compounds which tend to have varying functional groups. The oxidation is catalyzed through the formation of two molecules of water with the loss of electron from a single molecule of oxygen (Figure 4). It also catalyzes the oxidation of non-phenolic substrates that are less soluble and more stable (Gianfreda *et al*. 1999). Xenobiotic substances can be removed by microbial laccase and it produces polymeric products used for bioremediation processes.

Polyaromatic hydrocarbons (PAHs) are compounds with benzene rings arranged linearly. They are among the major contaminants of the environment (Li *et al*. 2010; Zeng *et al*. 2011). Owing to their persistent, carcinogenicity, mutagenicity and toxic nature, these pollutants and their derivatives pose severe threats to flora and fauna (Li *et al*. 2010). They are formed as a result of incomplete combustion of industrial wastes and fossil fuels. Due to poor degradation rate and low water solubility, they are regarded as xenobiotics (Ihssen *et al*. 2015). Polyaromatic hydrocarbons are converted to quinone form by microbial laccase and subsequently degraded to carbon dioxide (Khlifi *et al*. 2010). Textile dyes and phenols produced by the textile industry can also be detoxified and removed by laccases (Sondhi *et al*. 2008). Some of the applications of laccase reported include decolorization, degradation and detoxification of various components of distillery effluent as well as wastes from paper and pulp industries (Chandra and Chowdhary 2015).

### **Microbial Peroxidase**

Produced by plant and microorganisms, peroxidases 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. Their activity greatly depends on the presence of peroxides e.g. hydrogen peroxide, manganese peroxide, lignin peroxides and other peroxidases from diverse sources. The enzyme is first oxidized by the peroxides and subsequently, oxidation of the substrate is catalyzed by the oxidized enzyme. In the treatment of aqueous aromatic pollutants, peroxidases from various sources have been greatly exploited (Karam and Nicell 1997). Peroxidases are classified as haem and non-haemproteins (Koua *et al*. 2009).

Haem-peroxidases are found in animals, plants, fungi, and prokaryotes. They are further subdivided into Class I, II and III on the basis of sequence comparison. The Class I include the ascorbate, cytochrome and catalase peroxidases which are all intracellular enzymes. Class II includes the manganese (MnP) and lignin (LiP) peroxidases. They are produced by certain fungal species and their main function is the breakdown of plant lignin. Class III includes the horseradish peroxidases (HRP) from plant sources such as horseradish, soybean or barley and they catalyze the biosynthesis of plant cell wall and lignification reactions (Hiner *et al*. 2002).

The Non-haem peroxidases on the other hand are grouped into five non-related independent families. They include alkylhydro-peroxidase, manganese-catalase peroxidase, NADH peroxidase, non-haem haloperoxidase and the thiol peroxidases. The thiol peroxidases is the largest group with two sub-families: the peroxy redoxins and glutathione peroxidases (Koua *et al*. 2009). On account of activity, enzyme source and potential to naturally degrade toxic pollutants, peroxidases are also categorized into versatile peroxidase (VP), manganese-dependent peroxidase (MnP) and lignin peroxidase (LiP).

#### **Microbial Versatile Peroxidases:**

Due to their wide substrate specificity and oxidation in the absence if manganese, members of this group have been exploited in bioremediation of recalcitrant wastes and other industrial processes (Tsukihara *et al*. 2006; Wong 2009). Similar to manganese, lithium and Horesradish peroxidases, versatile peroxidases catalyze the oxidation of substrates such as Mn2+, phenolic aromatic compounds, phenolic and non-phenolic lignin dimers and methoxybenzene (Ruiz-Duenas *et al*. 2007).

#### **Microbial Manganese Peroxidases:**

The production of manganese peroxidase is stimulated by Mn2+, thereby acting as a substrate for the enzyme. Produced extracellularly by basidiomycetes class of fungi, manganese peroxidases are heme enzymes that catalyzes the oxidation of Mn2+ to Mn3+ in a series of reactions. The oxidant Mn3+ serves as an intermediary for phenolic compound oxidation. Due to its small size, the Mn3+ chelate oxalate diffuses into regions inaccessible to enzymes. Typical examples are lignin and xenobiotic pollutants buried deep into the soil and inaccessible to enzymes (Ten Have and Teunissen 2001).

#### **Microbial Lignin Peroxidases:**

These enzyme groups play an important role in breakdown of lignin, a plant cell wall component. Produced majorly as a secondary metabolite by the white rot fungi, lignin peroxidases are heme proteins that catalyzes degradation of lignin and other phenolic compounds in the presence hydrogen peroxide (H2O2) (co-substrate) and veratryl (mediator). The enzyme also catalyzes the oxidation of aromatic compounds but the mechanism of action is not well known (Piontek *et al*. 2001).

### **Microbial Dehydrogenase**

Microbial dehydrogenases are oxidoreductase found majorly in bacteria and yeast. Microbial alcohol dehydrogenase catalyzes the transformation of alcohols to yield aldehydes or ketones. They are grouped as Nicotinamide Adenine Dinucleotide (NAD+) or Nicotinamide Adenine Dinucleotide Phosphate (NADP+) -dependent dehydrogenases and NAD+ or NAD(P)+-independent dehydrogenase. The NAD+ and NADP+ - independent dehydrogenase use pyrroloquinoline, quinone, heme, or F420 as a cofactor (Chandrakant and Shwetha, 2011). In the same vein, aldehyde dehydrogenase catalyzes the NADP+-dependent transformation of aldehyde to carboxylic acid (Nickolas and Vasiliou 2003).Polyethylene glycol dehydrogenase from cell-free extracts was found to degrade polyethylene glycol and xenobiotics emitted from industries (Kawai and Yamanaka 1989).Secretion of NAD+-dependent polypropylene glycol dehydrogenase (PPG-DH) by *Stenotrophomonasmaltophilia* oxidizes hydrophobic polymers with medium chain secondary alcohols, di-propylene glycols, tri-propylene glycols and polypropylene glycols(Tachibana *et al*. 2008).

## Microbial Hydrolytic Enzymes

This significance of this category of enzymes is due to their exploitations in breakdown of waste biomass (Schmidt 2006) especially from food, agricultutral, chemical and biomedical industries. Microbial enzymes with hydrolytic activity employed in different waste treatment and recycling include cellulase and hemicellulase, protease, lipase, amylase, lactase, xylanase and pullulanase (Sanchez-Porro *et al*. 2003).

### **Microbial Cellulases**

Over the years, the treatment of agricultural wastes rich in cellulose, lignocellulose and related biomaterials using microbial enzymes is continuously gaining attention (Chandrakant and Shwetha 2011). The production of high value products such as bio-ethanol, biogas, enzymes, sugars from the conversion of agricultural and municipal wastes rich in cellulose and ligno-cellulose using microbial cellulases has increased the interests for application in industries (Sun and Cheng 2002). Microbial cellulase, glucanase, cellobiohydrolase, glucosidase, carbohydrate and cellobiase are continuously being exploited in the degradation and recycling of agricultural and municipal wastes. In order to meet the ever growing population, cellulases are continuously being exploited in food and feed production from the conversion of cellulosic waste resources (Bennet *et al*. 2002).

Cellulases produced by microorganisms could be intracellular or extracellular. Bacteria and fungi have been reported to liberate cellulases and related enzymes extracellularly but at very low levels (Adriano-Anaya *et al*. 2005). Species of *Bacillus* have been found to produce alkaline cellulases while fungi such as *Trichoderma* and *Humicola* are known to produce neutral and acidic cellulases. More often, cellulases consist of a combination of a number of enzymes especially from microbial sources. In the process of hydrolysis, three main groups are implicated; the Endoglucanse, Exoglucanase and beta-glucosidase. In the cellulose fibre, the sites of low crystallinity are first acted upon by the endoglucanase creating free chain ends. Thereafter, the cellulose molecule is further broken down by exoglucanase (cellobiohydolase) through cellubiose molecules elimination from the free chain ends. Finally, hydrolysis of cellubiose to glucose units is catalyzed by beta-glucosidase (Figure 5). In the enzymatic cellulose hydrolysis by cellulases to reducing sugars which are fermented by bacteria and yeasts to produce ethanol, the presence of some secondary enzymes along with the key enzymes has been reported (Sun and Cheng 2002).

Cellulases have found various applications especially in textile, brewing, paper and pulp industries. In the textile industry, cellulases have been exploited in the brightening of colour and softening of materials. Since cellulases catalyze the removal of cellulose microfibrils formed during washing, the enzyme has also been used in the manufacture of detergents. In the brewing industry, cellulases are employed in treatment of cellulose rich biomass to produce ethanol. Similarly, addition of the enzyme in fruit pulp increases the liberation of the juice. Cellulases have also been exploited in the paper and pulp industry for elimination of ink during paper recycling process.

### **Microbial Lipases**

Lipases are ubiquitous and can be isolated from a wide group of plants, animals and microorganisms, they are known to breakdown lipids to its corresponding monomers (free fatty acids and glycerol). The enzyme group has been reported to have a close relationship to soil organic pollutants. Thus, their activity was found to be accountable for the tremendous reduction in hydrocarbon pollutants present in soil. In industries, lipases from microbial sources are most commonly exploited than other sources of the enzyme. Some of the reactions catalyzed by these enzymes include alcoholysis, esterification, hydrolysis and aminolysis (Prasad and Manjunath 2011).

Lipolytic activities of lipases occur in a two-phase system i.e. the lipid-water interface (Figure 6), where the substrates of lipases appear to be stable between three states; the monomeric, the micellar and the emulsification states (Prasad and Manjunath 2011). Hence, two major groups of lipases have been reported based on the following factors (a) improvement of lipase activity following the immediate emulsification of triacylglycerides and (b) lipases with its active site containing a protein covering (lid) loop (Sharma *et al*. 2011).

Natural fats and oils contain triglycerides as the major component and the triglycerides can undergo hydrolysis to produce monoacylglycerol, diacylglycerol, free fatty acids and glycerol. These resulting products of hydrolysis are exploited for different purposes. In cosmetics, pharmaceutical and food industries, monoacylglycerols are greatly utilized as emulsifying agents. The activity of lipase is employed as a major valuable marker for assessing the degradation of hydrocarbon in polluted soil (Margesin *et al*. 1999).

The prospects of lipase in manufacturing, food, cosmetics, detergent, paper/pulp and chemical industries are enormous coupled with its potentials as indicators in biodegradation and bioremediation of contaminated soil. However, application of the enzyme is limited to the industry due to the high cost associated with the enzyme production (Sharma *et al*. 2011).

### **Microbial Proteases**

Their ability to hydrolyze peptide bonds of polymeric proteins in aqueous environment into different amino acid monomers (Figure 7) makes microbial proteases an important enzyme in food, leather, tannery and pharmaceutical industries (Singh 2003). Proteases hydrolyze proteinaceous substances which are introduced into the environment due to death of animals, shedding and moulting of body parts of animals, and protein-rich byproduct production from fishery, tannery, dairy, poultry and related industries. Depending on the nature of peptide chain catalysis, proteases are categorized as exopeptidases and endopeptidases (Beena and Geevarghese 2010).

Endopeptidases: sub-divided into metallo, cysteine, aspartic and serine endopeptidases depending on the location of the active site. The action of the endo-peptidases on the peptide chain is usually in the inner regions of the polymer. There is a negative impact on enzyme activity as a result of the free carboxyl and amino terminals from cleavage of the peptide bonds (Beena and Geevarghese 2010).

Exopeptidases: the activity of this group is close to the terminal carboxylic or amino sites of the chain. The aminopeptidases and the carboxylpeptidases then respectively act on the free amino and carboxyl terminals (Singh 2003).

Proteases have found applications in detergents, leather, pharmaceutical, and food production/processing industries. In the leather industry, animal skin processing for removal of hairs and other parts of the skins, alkaline proteases are used to achieve this. Also, in dipeptide aspartame production (an artificial non-calorie sweetener), the use of protease has been reported (Rao *et al*. 1998).

### **Microbial Amylases**

Microbial amylases are hydrolases of polysaccharide and they have found use in instantaneous saccharification, fermentation of starch and ultimately treatment of food wastes rich in starch (Shoemaker 1986; Karam and Nicell 1997). Amylases have also been employed in the production of alcohol using rice processing wastewaters as substrates (Shoemaker 1986; Karam and Nicell 1997). The enzyme has also been found to improve the treatment of activated sludge wastewaters through the decrease in treatment time. Another interesting application of this enzyme is in the consortium of alpha amylase and glucoamylase to convert starch-rich wastes in potato or cheese whey from food processing industries to produce biodegradable and photodegradable plastics (Coleman 1990; Karam and Nicell 1997). Utilization of α- amylase to cleave long molecules of starch into smaller fragments is initially carried out. Glucoamylase is further used to attack these small fragments producing glucose through saccharification of over 90% of the starch. Lactic acid bacteria subsequently act on the resulting glucose, producing lactic acid. Finally, recovery, purification and successive use of the lactic acid in production of environmentally friendly plastics are carried out. Proper combinations of isomers of lactic acid alongside other compounds usually control the rate of decomposition of the plastics (Coleman 1990; Karam and Nicell 1997).

## Other Enzymes

Many other enzymes from microbial sources have found use in waste recycling. Pectin lyase and Pectinesterase from *Clostridium beijerinckii* and *Clostridium thermosulfurogenes* respectively have been exploited in pectin degradation. The processing of food wastes such as apple pomace has been utilized to produce butanol (Blasheck 1992). *Candida norvegensis*, a yeast found to produce L-galactonolactone oxidase, an enzyme employed in the manufacture of L-ascorbic acid by biotransformation of excess galactose, a product of lactose hydrolysis of whey (Shoemaker 1986). Lactases have also been utilized in the recycling of dairy wastes rich in lactose and whey proteins to produce value-added products (Blasheck 1992; Karam and Nicell 1997).

Chitinase isolated from *Serratia marcescens* with the capability to degrade chitin has been reported. An alternative disposal of high chitin content contained in shellfish waste through the bioconversion to single-cell proteins was proposed. The method requires pretreatment of shrimp waste by first reducing the size, then elimination of proteins and minerals to give rise to a chitin substance which can easily be transformed by the action of chitinase to N-acetyl glucosamine, a substrate for production of single cell protein (Cosio *et al*. 1982). Table 1 presents a summary of microbial enzymes, sources and applications.

Table 1: Outline of some Enzymes and their Functions in Waste Recycling

|  |  |  |
| --- | --- | --- |
| Enzyme  | Microbial Source | Function |
| Alkylsulfatases | Bacteria  | Surfactant degradation |
| Amylases e.g.α and β - amylases Glucoamylase | Bacteria and Fungi | Starch hydrolysis and production of glucose |
| Cellulolytic enzymes e.g.Cellobio-hydrolase, Cellobiase, Cellulase, andExo-1,4-b-D-glucosidase  | Bacteria and Fungi | Sugar, alcohol and bio-energy production by hydrolysis of cellulose-rich sludge from paper, pulp and municipal solid wastes. |
| Chitinase | *Serratia marcescens*  | N-acetyl glucosamine productionfrom bioconversion of shellfish waste. |
| Chloro-peroxidase  | *Caldariomyces fumago* | Phenolic compounds oxidation |
| Cyanidase | *Alcaligenes denitrificans* | Decomposition of cyanide |
| Cyanide hydratase | Fungal e.g. *Gloeocercospora sorghi**Stemphylium loti* | Cyanide hydrolysis |
| Dehalogenase | Bacterial  | Bioremediation and transformation of halogenated organic compounds.Debrominate and mineralize Tribromophenol |
| L-Galactono-lactone oxidase | *Candida norvegensis* | L-ascorbic acid production from hydrolysis of galactose present in whey.  |
| Laccase  | Several fungi and bacteria | Binds aromatic amines and phenols to humus, elimination of phenols, decolorize effluents from Kraft bleaching  |
| Beta-galactosidase | Bacterial and Fungal | Processing of dairy wastes and subsequent production of high value products |
| Lignin peroxidase  | *Phanerochaete chrysosporium* | Decolorization of effluents from Kraft bleaching industries, elimination of phenols and other aromatic waste constituents. |
| Lipase | Bacteria and Fungi  | Enhanced dewatering of sludge  |
| Lyzozyme | Bacteria | Enhanced dewatering of sludge |
| Manganese peroxidase  | *P. chrysosporium* | Oxidize aromatic dyes and monoaromatic phenols. |
| Oxygenases:MonooxygenaseDi-oxygenase  | Bacterial and Fungal | desulfurization, dehalogenation, hydroxylation, denitrification, ammonification,biotransformation, bioremediation and biodegradation of various aliphatic and aromatic compounds. |
| Parathion hydrolase | *Pseudomonas* sp.*Flavobacterium**Streptomyces* | Hydrolysis of organophosphate in pesticides |
| Pectin Lyase  | *Clostridium beijerinckii*  | Pectin degradation |
| Pectinesterase  | *Clostridiumthermosulfurogenes* | Pectin degradation |
| Peroxidase | Bacterial  | Removal of phenols and aromatic amines, dewatering of sludge, decolorization of effluents from Kraft bleaching |
| Phosphatase | *Citrobacter* sp. | Heavy metal removal |
| Proteases | Bacterial and Fungal  | Sludge improvement, Digestion of meat and fish wastes. |

Source: (Karam and Nicell 1997; Chandrakant and Shwetha 2011)

# SIGNIFICANCE OF MICROBIAL ENZYMES IN WASTE RECYCLING

The role microbial enzymes play in recycling of different varieties of waste cannot be overemphasized. Enzymatic bioconversion of wastes has the dual benefit of decreasing the quantity of otherwise worthless materials to be disposed and subsequently create products of significant value such as food, feed, bio-fuels or other bio-products. Conventional chemical and biological processes of efficient treatment, reduction or removal of these waste materials from the environment have proved hard to achieve. Hence, enzymatic processing of wastes which falls between these two traditional classes have shown significant bioremediation potentials due to the following merits: faster and cheaper operation through a broad variety of factors such as pH, temperature, salinity etc); application to a wide variety of wastes; function at low and high concentrations of contaminants; nonexistence impediments regarding biomass adaptation; shock load effects are non-existent; reduction in sludge quantity and also the ease in the control of the processes involved in waste treatment.

#  LIMITATION OF MICROBIAL ENZYMES IN WASTE RECYCLING

Regardless of the immense potentials microbial enzymes offers, some limitations still linger. Some of which are; cost of enzymatic treatment/recycling of wastes; selection of the most suitable microbial enzyme or in some cases group of enzymes which is also a function of the enzyme specificities; the need for cofactors of some enzymes; the ability of enzymes to retain their active nature under normal conditions of operation for a prolonged period of time; and difficulty in assessing the toxicity and subsequent disposal of enzymatic reaction by-products (Karam and Nicell, 1997).

#  FUTURE PROSPECTS

Environmental pollutants have serious health hazard on humans, animals, plants and other life forms in nature, with various destructive effects of such as respiratory disorders, cardiovascular disorders, cancer, allergic reactions, mental disorders, perinatal disorders and even mortality, as such recycling of these wastes is immensely significant. The acknowledgment of the wide potentials microbial enzymes exhibits in recycling of the different sources and forms of wastes is well known. Nonetheless, synergies between microbial enzymes are currently being recognized as an effective strategy for bio-product development from waste biomass. In order to economically and sustainably recycle waste to produce useful bio-products, more extensive use of the microbial enzyme omics technologies, such as genomics, metabolomics, transcriptomics, proteomics, and interactomics should be encouraged. Application of these molecular studies in the efficient enzymatic breakdown of waste materials will go a long way in providing a better understanding of individual and interactive roles of the vast microbial enzymes in the degradation, biotransformation and ultimately, by-products creation and valorization.

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