UNIT 5 DEGRADATION OF NATURAL COMPOUND

Structure

5.1 INTRODUCTION

Biodegradation is biological catalyzed reduction in complexity of chemical compounds. It is the process by which organic substances are broken down into smaller compounds by living microbial organisms (Rani et al., 2008). When biodegradation is complete, the process is called "mineralization". However, in most cases the term biodegradation is generally used to describe almost any biologically mediated change in a substrate (Brunner, 2014).

The microbial organisms transform the substance through metabolic or enzymatic processes. Several microorganisms, including fungi, bacteria and yeasts are involved in biodegradation process. Final product of the degradation is carbon dioxide. Organic material can be degraded aerobically, with oxygen, or anaerobically, without oxygen. Certain environmental factors such as temperature, pH, and available nitrogen and phosphorus sources, then, seem to determine the rate and the extent of degradation (Gupta et al., 2013).

5.2 OBJECTIVES

After reading this unit you should be able to:

- Explain Biodegradation.
- Describe the degradation of cellulose.
- Describe the many aspects of hemicellulose degradation.
- Describe the Lignin and its degradation.
- Explain the Lignocellulolytic enzymes
- Explain Composting and Vermicomposting
- Explain mushroom Cultivation by using agro residues.

5.3 DEGRADATION OF CELLULOSE

Definition of cellulose

Cellulose is an organic polysaccharide composed of a linear chain of hundreds of β-linked D-glucose units. Cellulose, a linear polymer of D-glucose units (two are shown) linked by β(1→4)-glycosidic bonds (figure 1)

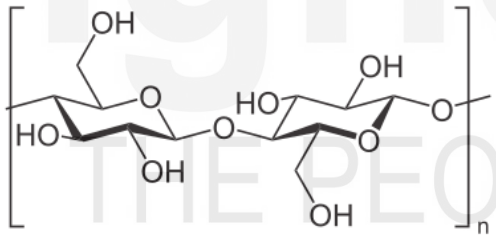


Figure 1: Structure of cellulose (Source: Xiros et al., 2011)

Cellulose is the most abundant extracellular structural polysaccharide or organic polymer of all biomolecules in the biosphere. Cellulose is present in all land plants but is completely lacking in meat, egg, fish, and milk. It is, however, not metabolized by the human system. It is the most widely distributed carbohydrate of the plant kingdom that comprises about 50% of all the carbon in vegetation (Brunner, 2014).

Cellulose is a homopolymer of a glucose derivative, and thus, it acts as a great source of fermentable sugar. Cellulose is cultivated in the form of energy crops for the production of ethanol, ethers, acetic acid, etc. The abundance of cellulose is due to the constant photosynthetic cycles in higher plants, synthesizing about 1000 tons of cellulose. Cellulose is a fibrous, rigid, white solid, insoluble in water but soluble in ammonical cupric hydroxide solution. Although insoluble in water, cellulose absorbs water and adds to the bulk of the fecal matter, and facilitates its removal (Xiros et al., 2011). Structure of cellulose is shown in figure 1.

Microbial degradation of cellulose

The molecular weight of cellulose ranges between 200,000 and 2,000,000. Cellulose consists of a D-glucose unit at one end with a C4-OH group as the non-reducing end, and the terminating group is C1-OH as the reducing end. The bond is formed by taking out a molecule of water from the glycosidic OH group on carbon atom 1 of one β-D-glucose molecule and the alcoholic OH group on carbon atom 4 of the adjacent β-D-glucose molecule. Anhydrocello biose is the repeating unit of cellulose. Cellulose has great tensile strength and low accessibility. Most of the cellulose found in wood is highly crystalline with about 65% crystalline regions (Brunner, 2014).

Microorganisms involved in cellulose degradation (cellulolytic microorganisms)

A broad spectrum of cellulolytic microorganisms, mainly fungi, and bacteria, have been identified over the years (Brunner, 2014). The structure and mode of action of the cellulases produced by different microorganisms are also different.

Cellulolytic Fungi

Cellulase-producing fungi are widespread among fungi and include species from the ascomycetes (Trichoderma reesei), basidiomycetes (Fomitopsis palustris) with few anaerobic species.

- **Soft rot:** *Trichoderma Aspergillus niger*, *Fusarium oxysporum*, *Neurospora crassa*, etc.
- **Brown rot and:***Poria placenta*, *Lenzites trabea*, *Coniophora puteana*, and *Tyromyces palustris*.
- **White rot fungi:***Phanerochaete chrysosporium*, *Sporotrichum thermophile*, and *Trametes versicolor*.

Cellulolytic Bacteria

Most of the bacterial cellulolytic enzymes are *Bacillus*, *Acinetobacter*, *Cellulomonas*, *Fibrobacter succinogenes*, *StaphylococcPseudomonas, Proteus Ruminococcus albus* and *Clostridium*. Some thermophilic bacteria like *Anoxybacillus sp*, *Geobacillus sp*, and *Bacteroides* also exhibit cellulase activity.

Process (Simple Steps) of cellulose degradation

Cellulose degradation occurs in three simple steps;

1. **Hydrolysis by endoglucanases**

   The first step in the degradation of cellulose is the action of endoglucanases that randomly attack the cellulose fibrils. This step results in a decrease in the size of cellulose chains as it degrades the polymer into smaller fragments. The enzyme acts internally at random

points of the polymer.

2. Hydrolysis by exoglucanases

Exoglucanases act on the smaller fragments resulting in even smaller units of tetrasaccharides or disaccharides. Exoglucanases act on the reducing end of the fragments to form either dimeric units or cellobiose.

3. Hydrolysis by β-glucosidase

β-glucosidase or cellobiose act on the dimeric units of glucose of cellobiose to form monomeric units, glucose. This is the final step of cellulose degradation that results in the formation of free individual units of the glucose molecule. (López-Mondéjar, 2016)

Schematic diagram has been shown in figure 2

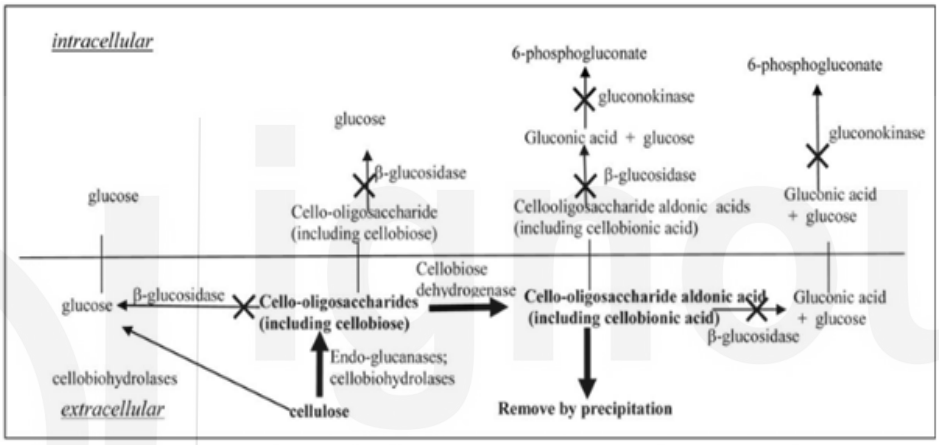


Figure 2: Simple Steps of cellulose degradation (Source: Xiros et al., 2011)

5.4 DEGRADATION OF HEMICELLULOSE

Hemicellulose is a group of complex polysaccharides that are found in the fibers of plants along with other polysaccharides like cellulose and pectin. Hemicelluloses are mostly mixed polymers, whereas cellulose is a homopolymer of glucose. Apart from arabinogalactan, all other hemicelluloses have short side-chains and low molecular weights. The hemicelluloses consist of either pentose (xylose, arabinose) or hexoses (glucose, mannose, galactose) as well as uronic acids. These polysaccharides adsorb water and function as storage and supporting substances in plants.

Hemicelluloses are grouped into groups that include xyloglucan, xylans, mannans and glucomannans, and β-(1→3, 1→4)-glucans. These glycans all have the same equatorial configuration at C1 and C4, and hence the backbones have significant structural similarity. Xylan is the representative polysaccharide of this group that is composed of a backbone of β-(1→4)-linked xylose residues. Xylans are also the most abundant carbohydrates after cellulose (Scheller and Ulvskov, 2010).

Structure of hemicellulose

Hemicelluloses consist of 50–3000 sugar units as opposed to 7000–15,000 glucose molecules per polymer in cellulose. Hemicellulose is amorphous in

structure, not crystalline as cellulose, and therefore more susceptible to hydrothermal extraction and hydrolysis. Hemicelluloses are classified into different groups as xylans, mannans, and glucans on the basis of the primary sugar residue in the backbone. Structure of hemicellulose is represented in figure 3.

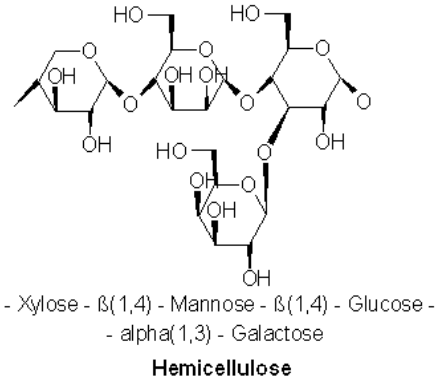


Figure 3: Structure of hemicellulose (Source:Scheller and Ulvskov, 2010)

1. **Xylans**

   Xylans are a group of polysaccharides consisting of β-(1→4)-linked xylose sugar residues with side branches of α-arabinofructose and α-glucuronic acids that contribute to the cross-linking of cellulose. Xylans are categorized into three classes; glucoronoxylan, arabinoxylan, and glucoronoarabinoxylans.

2. **Mannans**

   Mannans are a group of β-(1→4)-linked polysaccharides where the backbones consist entirely of mannose units.

3. **Glucans**

   Glucans are polysaccharides composed of glucose units linked by glycosidic bonds. Glycans in hemicellulose are either xyloglucans or β-(1→3,1→4)-glucans.

Hemicellulases

Hemicellulases are categorized into four different groups depending on the substrate and linkages they act on; L-arabinanases, D-galactanases, D-mannanases, and D-xylanases. Some of these enzymes might even differ in structure and their mode of action. Different hemicellulases might even be produced by a different group of organisms like bacteria, fungi, protozoans, algae, and plants.

Microorganisms involved in hemicellulose degradation

1. **Hemicellulolytic fungi**

- *Alternaria solani*, *Botryosphaeria ribis*, *Botrytis allii*, *Corticium centrifugum*, *Monilia fructigena*, *Neurospora, Penicillium digitatum*, *Rhizopus nigricans*, *Sclerotinia fructigena*, etc. are known to produce L-arabinanases and D-mannanases.

- Similarly, fungi like *Gibberella saubinetti*, *Helminthosporium oryzae*, *Phytophthora infestans*, *Trametes gibbosa*, etc. produce D-galactanases.

2. Hemicellulolytic bacteria

Most of the bacterial cellulolytic enzymes are *Clostridium felsineum*, *Bacillus subtilis*, *Acetenobacter mannanolyticus*, *Bacillus aroideae*, *Sporocytophaga myxococcoides*, etc.

Factors affecting hemicellulose degradation

Degradation of hemicelluloses is affected by a number of factors, some of which are temperature and pH (pH 6 and 40°C), organic matter,dosage of enzymes, Substrate conversion

Process (Simple Steps) of hemicellulose degradation

- The degradation begins with the attack by exoglycocides on the hemicelluloses in order to remove the side-chain substituents, hereby, 'opening up' or exposing the backbone glycan chain.
- This first step allows the glycan chain to be exposed so that it can be easily attacked by the hemicellulases, as a steric hindrance by the side chain residues is reduced.
- Alternatively, the degradation begins with the attack of endohemicellulases on the regions of glycan chain that are unbranched or relatively moderately branched by substituents.
- It is then followed by the action of endohemicellulases which yields an array of oligosaccharides of a mixed constitution.
- Both exoglycosidases and endohemicellulases further degrade the resulting fragments. (López-Mondéjar, 2016)

Schematic diagram of hemicellulose is shown in figure 4.

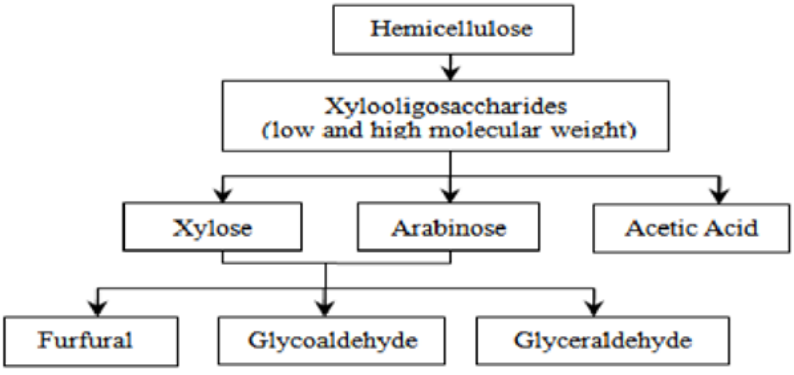


Figure 4: Hemicellulose Degradation (Source:Scheller and Ulvskov, 2010)

5.5 DEGRADATION OF CHITIN

Chitin is a complex homopolysaccharide consisting of units of amino sugar glucosamine that accounts for the second most abundant polysaccharide of

nature after cellulose. The term 'chitin' is derived from the Greek word 'chiton' which means a coat of mail. Depending on the source, chitin occurs in two forms; α and β conformation. A third less discussed γ form is also known. These allomorphs differ from one another in their orientation of the micro-fibrils. Chitin is considered an essential polymeric structure due to its characteristics like high porosity, biodegradability, predictable degradation rate, and structural integrity. Chitin degradation in soil or on artificial media can be affected by several factors, some of which areMoisture content, added glucose, Aeration and Organic matter.

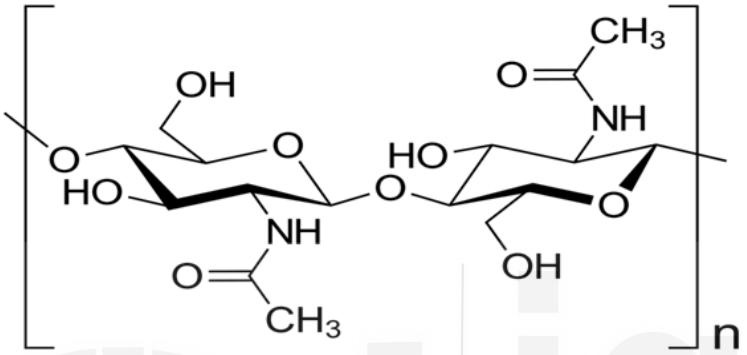


Figure 5: Structure of Chitin (Moussian, 2019)

Structure of chitin

Chitin exists as a linear polymer of N-acetyl-D-glucosamine units linked together by β-1,4-glucosidic linkages. This structure results in a three-dimensional α-helix configuration. The stability of the α-helix chitin structure is brought about by the hydrogen bonding of the N-acetyl side chains. In nature, however, the chitin polymers bind extracellularly by intermolecular hydrogen bonding that forms a crystalline microfibril structure. Structure of chitin is shown in figure 5.

Microorganisms involved in chitin degradation

1. **Chitinolytic bacteria**

   Bacterial species of *Vibrio*, *Photobacterium*, *Aeromonas*, *Cytophaga*, *Streptomyces*, *Photobacterium*, *Bacillus*, *Clostridium*, and *Chromobacterium* are well-known chitinolytic bacteria.

2. **Chitinolytic fungi**

   The most common fungal species involved in chitinolysis include Mucorales like *Mortierella spp*, and Deuteromycetes and Ascomycetes like *Aspergillus*, *Verticillium*, *Thielavia*, *Trichoderma*, *Penicillium*, and *Humicola*.

3. **Slime mold, protozoa, and algae**

   Myxomycetes (true slime molds) like *Physarum polycephalum* are a rich source of lytic enzymes that produce a complex of extracellular chitinases. Soil protozoa like Hartmanella and Schizopyrenus, along with

slime mold Plasmodium are also known to produce chitinases that participate in the digestion of chitinous food particles engulfed by these invertebrates (Moussian, 2019).

Process (Simple Steps) of chitin degradation

The hydrolysis of chitin occurs in a two-step process;

1. **Depolymerization**

   Depolymerization is the process of reduction of chitin polymer length by the breakdown of β-1,4 linkages between the N-acetyl glucosamine units. This process results in the release of N-acetylglucosamine units by the action of chitinases of chitosanases.

2. **Deacetylation**

   Depolymerization is followed by acetylation which causes the release of glucosamine units and acetic acid. Chitin deacetylases act on the N-acetyl glucosamine dimer or trimers, resulting in the catalytic degradation of the larger molecule into smaller ones. The end products of this step are glucosamine and acetic acid, which are then utilized by the microorganism for various purposes.

   Microbial chitin degradation occurs by one of the two mechanisms; chitinoclastic mechanism and deacetylation mechanism.

1. **Chitinoclastic**

   In this mechanism, the substrate is acted upon by the chitinolytic system, consisting of chitinases. Exochitinase breakdown acetylchitobiose units from the non-reducing end of the polysaccharide chain. Endochitinase cleaves glycosidic linkages randomly along the chain, eventually resulting in the formation of diacetylchitibiose as the major product, along with some tri-acetyl chitotriose (Casadidi et al., 2019).

2. **Deacetylation**

   The group of enzymes involved in the deacetylation mechanism is termed deacetylases. These enzymes catalyze the process of deacetylation of N-acetylglucosamine polymer. The hydrolysis of chitosan occurs in the presence of chitosanases that breakdown the linkages between the β-glucosamine units linked together by β-1, 4-glycosidic linkages. This cleavage results in the release of chitobiose (glucosaminyl-(1-4)- β-glucosaminide). Degradation of Chitin is shown n figure 6.

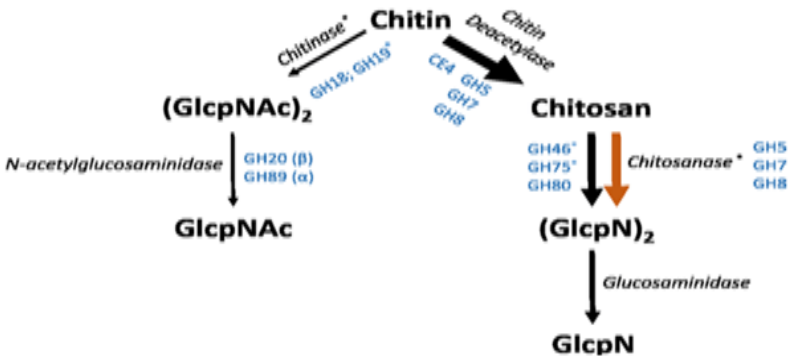


Figure 6: Degradation of Chitin (Moussian, 2019)

Check Your Progress 1

Note: a) Use the space given below for your answers.

b) Check your answers with those given at the end of the unit

1. What is Biodegradation?

   ……

   ……

   ……

   ……

   ……

2. Explain degradation of cellulose.

   ……

   ……

   ……

   ……

   ……

3. Define Chitin and its degradation?

   ……

   ……

   ……

   ……

   ……

5.6 DEGRADATION OF LIGNIN

Lignin is a It is the most abundant aromatic biopolymer that consists of about 30% of the organic carbon on Earth. Lignin is an essential component of the lignocellulosic biomass where it accounts for about 15-30% of the total weight (Rani et al., 2008).

The chemical species like hydroxycinnamyl alcohols (or monolignols) coniferyl alcohol and sinapyl alcohol, with typically minor amounts of p-coumaryl alcohol, are the primary building blocks of lignin.

Structure of lignin

Lignin is composed of phenylpropane units linked together by the chemical linkages of alkyl-alkyl, alkyl-aryl, and aryl-aryl groups. Ecological factors, like plant growth, nutrition, climate, and illumination, also affect the chemical structure of lignin.

Natural lignin consists of three important elements: carbon, hydrogen, and oxygen, of which the carbon content is much higher than in homogenous carbohydrates. Besides, important structural characteristics of lignin include the functional groups, including alcohol hydroxyl group, carbonyl group, carboxyl group, phenolic hydroxyl group, methoxyl, and sulfonic acid. Structure of lignin is shown in figure 7.

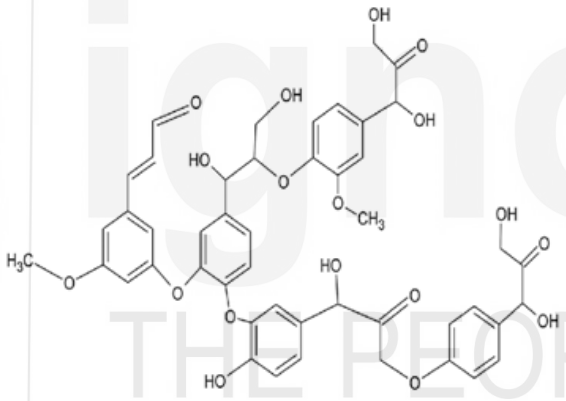


Figure 7: Structure of Lignin (Source: Vinardell et al., 2017)

Microorganisms involved in lignin degradation

Different microbial population dominates at various stages and has distinct roles in the degradation of organic matter.

1. **Lignin-degrading bacteria**

   The occurrence of lignin-degrading enzymes has been observed in *Mycobacterium tuberculosum*, *M. avium*, *Pseudomonas syringae*, *P. aeruginosa*, *P. putida*, *Bordetella pertussis*, *Xanthomonas campestris*, *Escherichia coli*, *Caulobacter crescentus* *Rhodobacter capsulatus*, *Yersinia pestis*, *Campylobacter jejuni*, and *Aquifex aeolicus*.

2. **Lignin-degrading Actinomycetes**

   Streptomyces and other actinomycetes have been identified as lignin-degrading species and can be isolated from a wide variety of sources, including a range of soils, high-temperature environments, and termite guts. Lignin-degrading enzymes have been observed in five different species of Streptomyces; *Streptomyces antibioticus*, *S. griseus*, *S.*

coelicolor, *S. cyaneu*s, and *S. lavendulae*.

3. Lignin-degrading Fungi

Among different wood-decaying fungi, only the white rots have the potential to degrade all three major components of wood entirely. These fungi mainly belong to the Ascomycetes, Deuteromycetes, or Basidiomycetes group. Typical examples of white-rot fungi are *Ganoderma applanatum* and *Heterobasidion annosum* that preferentially remove lignin without a substantial loss of cellulose and cause white-pocket or white-mottled type of rot. Other Ascomycetes like *Rhizoctonia solani*, *Aspergillus nidulans*, *Podospora anserina*, *Neurospora crassa*, *Gaeumannomyces graminis var. tritici* and *Trichoderma reesei* have also been described to produce laccase and other lignocellulolytic enzymes.

Lignolytic Enzymes

Primarily, three different enzymes are involved in lignin degradation; manganese peroxidase (MnP), lignin peroxidase (LiP), and laccases..

1. Lignin peroxidases (LiP)

Lignin peroxidase is an extracellular heme-containing peroxidase that is dependent on H_2O_2 and degrades a variety of lignin-related compounds. These enzymes have an unusually high redox potential and low optimum pH, typically showing little specificity toward substrates (Rani et al., 2008).

Lignin peroxidase is well known as part of the ligninolytic system both of aphyllophoralic and agaricalic fungi. These peroxidases preferably oxidize methoxylated aromatic ring without a free phenolic group. Methoxylated benzenes and benzyl alcohols are the simplest aromatic substrates for lignin peroxidase. The role of lignin peroxidase in ligninolysis could be the further transformation of lignin fragments which are initially released by manganese peroxidase.

LiP is used commercially to mineralize a variety of recalcitrant aromatic compounds, like three- and four-ring polyaromatic hydrocarbons, polychlorinated biphenyls, and natural dyes.

2. Manganese peroxidases (MnP)

Manganese peroxidase is an extracellular heme-containing peroxidase with a requirement for Mn^{2+} as its reducing substrate that has lignin-reducing properties. Manganese peroxidase is one of the most common lignin-degrading peroxidases produced by the majority of wood-decaying fungi and many litter-decomposing fungi. Structurally, these enzymes are glycosylated proteins with an iron protoporphyrin IX (heme) prosthetic group.

The enzyme oxidizes Mn^{2+} to Mn^{3+},which in turn oxidizes phenolic structures to phenoxyl radicals. Mn^{3+} formed is highly reactive and

complexes with chelating organic acids such as oxalate or malate. As the redox potential of the MnP-Mn complex is lower than that of lignin peroxidase, it preferably oxidizes phenolic substrates. The phenoxyl radicals formed might further react, resulting in the eventual release of CO_2. The phenoxyl radicals formed subsequently cleave Cα -Cα or alkyl-phenyl bonds causing depolymerization to smaller intermediates including quinones and hydroxyl quinines.

3. Laccases

Laccases are a group of lignin-degrading enzymes consisting of N-glycosylated extracellular blue oxidases and four copper atoms in the active site that are distributed among different binding sites. Laccases catalyze the oxidation of several aromatic hydrogen donors with subsequent reduction of oxygen to water.

Moreover, laccases oxidize not only the phenolic and methoxyphenolic acids but also decarboxylate them and attack their methoxy side chains or groups. Several fungal laccases have been considered for the oxidation of compounds like 1-(3,4-dimethoxyphenyl)-2-(2-methoxyphenoxy) propan-1,3-diol (I), and phenolic lignin model compounds like phenol red in the presence of the redox mediators. Laccases have been reported to oxidize many recalcitrant substances, such as chlorophenols polycyclic aromatic hydrocarbons (PAHs), lignin-related structures, and organophosphorus compounds (Rani et al., 2008).

Process (Simple Steps) of lignin degradation

The overall process of lignin degradation can be explained in two simple steps;

1. Depolymerization

The first step of lignin degradation is the depolymerization of aryl and biaryl compounds such as β-aryl ethers. This step is a non-specific step that occurs extracellularly by different bacterial and fungal enzymes.

Depolymerization of lignin occurs as a result of cleavage of β-O-4 ether bond that represents about 50% or more of the total linkages in lignin. This step decreases the length of the lignin polymer so as to obtain dimeric or oligomeric units, which can then further be degraded into smaller molecules. Depolymerization in microorganisms is catalyzed by different lignin-degrading enzymes found in many microorganisms like peroxidases and phenol oxidases. These enzymes attack the lignin randomly, then convert the phenolic group to free-radicals, and these radicals lead to lignin depolymerization(Vinardell et al., 2017).

2. Solubilization and Mineralization

The smaller molecules of lignin formed after depolymerization are now taken by different microorganisms that catalyze a series of conversion by various in vivo enzymes. Most of the linkages within the lignin

molecules have their specific metabolic pathways to cleave these specific linkages. The mineralization and solubilization of oligomers and monomers result in the formation of CO2 and other essential molecules that can be utilized by the organisms. Degradation of lignin is shown in figure 8.

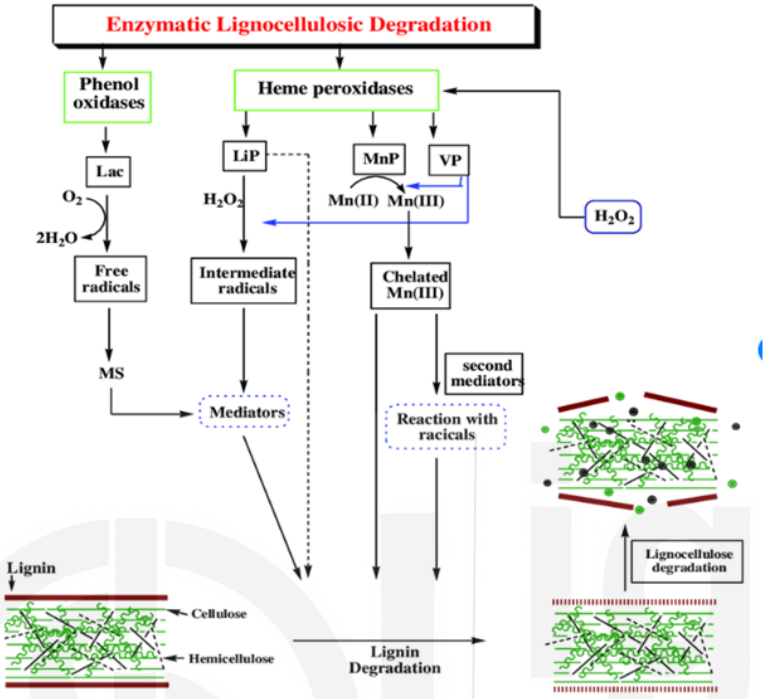


Figure 8: Degradation of Lignin (Source: Vinardell et al., 2017)

5.7 ENVIRONMENTAL FACTORS INFLUENCES IN BIODEGRADATION

Soil type and soil organic matter content affect the potential for adsorption of an organic compound to the surface of a solid. Absorption is an analogous process wherein a contaminant penetrates into the bulk mass of the soil matrix. Both adsorption and absorption reduce the availability of the contaminant to most microorganisms and the rate at which the chemical is metabolized is proportionately reduced. Variations in porosity of the unsaturated and saturated zones of the aquifer matrix may influence the movement of fluids and contaminant migration in groundwater. The ability of the matrix to transmit gases, such as oxygen, methane and carbon dioxide, is reduced in fine grained sediments and also when soils become more saturated with water. This can affect the rate and type of biodegradation taking place. The oxidation-reduction potential of a soil provides a measurement of the electron density of the system. Biological energy is obtained from the oxidation of compounds in which electrons are transferred to various more oxidized compounds referred to as electron acceptors. A low electron density (Eh greater than 50 mV) indicates oxidizing, aerobic conditions, whereas high electron density (Eh less than 50 mV) indicates reducing, anaerobic conditions

5.8 COMPOSTING

Composting is the natural process of turning organic matter in waste into a beneficial fertilizer that can benefit both soil and plants. Composting converts organic waste such as food waste, manure, leaves, grass trimmings, paper, wood, feathers, agricultural residue, etc. into beneficial organic fertilizer by using various microorganisms such as bacteria and fungus (Ipek et al., 2002).

Compostable materials include anything that can be consumed or cultivated in a field or garden. Compostable materials include fruits, vegetables, dairy products, cereals, bread, unbleached paper napkins, coffee filters, eggshells, meats, and newspapers. Plastics, grease, glass, and metals, such as plastic utensils, condiment packages, plastic wrap, plastic bags, foil, silverware, drinking straws, bottles, polystyrene, and chemicals, cannot be composted. Red meat, bones, and small bits of paper can be composed, but they decompose more slowly.

Composting goes through three main phases under optimal conditions:

Mesophilic Phase: It is an initial phase where mesophilic bacteria such as Listeria monocytogenes, Staphylococcus aureus, and Escherichia coli decompose the material at moderate temperatures.

Thermophilic Phase: As the temperature rises, a second, thermophilic phase begins, in which diverse thermophilic bacteria such as lactobacilli, Streptococcus thermophilus and bifidobacteria carry out breakdown at higher temperatures (50 to 60 °C).

Maturation Phase: In the maturation phase, when the supply of high-energy molecules diminishes, the temperature begins to drop, and mesophilic bacteria once again take the lead.

Procedures

Sorting and Shredding

Sorting and separating procedures isolate decomposable materials from glass, metal, and other inorganic components in trash. These are done mechanically, based on variances in the refuse's physical features such size, density, and magnetic properties. The size of the waste items is reduced by shredding or crushing, resulting in a homogenous mass of material. Hammer mills and rotary shredders are used to accomplish this.

Digesting and Processing

Composting crushed waste can be done in an open windrow or in an enclosed mechanical facility. It may take five to eight weeks for the waste to be completely digested, depending on moisture conditions. Temperatures in an active compost pile reach around 65 °C (150 °F) due to the metabolic action of aerobic bacteria, destroying harmful organisms that may be present in the waste material (Ipek et al., 2002).

Schematic Layout of Composting is shown in figure 9.

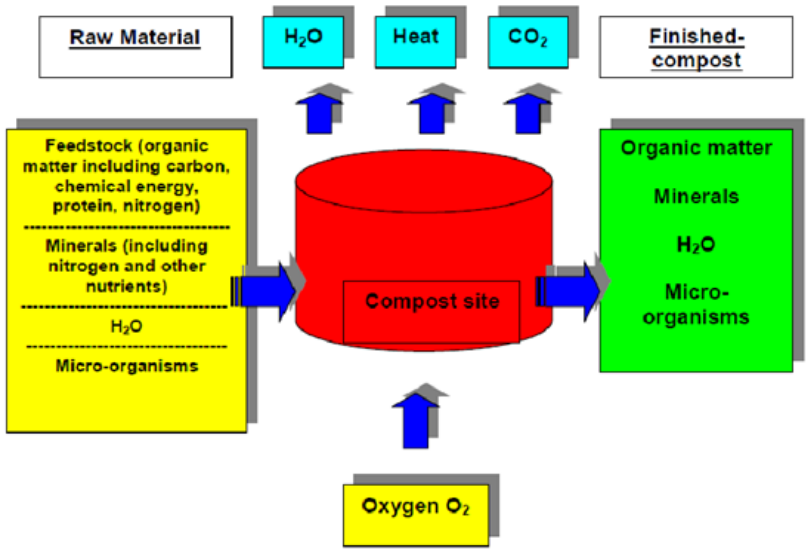


Figure 9: Schematic Layout of Composting (Source: Kumar 2011)

Various Methods

Passive Composting or Piling includes simply stacking the materials and allowing them to degrade naturally is passive composting or piling. This process is simple and inexpensive, but it is slow and may produce unpleasant odors. Various methods are described below

- **Aerated Static Piles:** Air is added to the stacked pile via perforated pipes and blowers in aerated static piles. This approach does not involve any labor to change the compost, but it is weather-dependent and can result in unpredictable pathogen elimination due to poor mixing.
- **Windrows:** Windrows are long, narrow piles that are turned when temperature and oxygen requirements dictate. This process generates a consistent output that can be remotely tracked. Turning the compost, on the other hand, might be time-consuming or expensive. Windrows are commonly utilized for huge volumes that take up a lot of space. Windrows can also cause odor issues and leachate concerns if they are exposed to rain.
- **Bins:** Small amounts of food waste are usually composed in bins. Bins with wire mesh or wooden frames allow for adequate air circulation and require little labor.
- **In-vessel System:** In-vessel systems, which use perforated barrels, drums, or specifically manufactured containers, are easy to use, turn, require little effort. They are not weather sensitive, and can be used in urban and public settings. The initial investment can be expensive, yet handling volumes are usually minimal. (Ipek et al., 2002).

Advantages

- Reduction in Methane

- Reduction in Chemical Fertilizers
- Compost encourages agricultural crop yields to increase.
- By rehabilitating contaminated, compacted, and marginal soils, compost can help with reforestation, wetlands restoration, and habitat rejuvenation projects.
- Compost can be used to rehabilitate soils that have been contaminated by hazardous waste at a low cost.
- Compost can save money over traditional soil, water, and air pollution remediation solutions.
- Improves Moisture Dispersion
- Aids Carbon Sequestration

5.9 VERMICOMPOSTING

The term vermiculture refers to the cultivation or production of earthworms. Vermicomposting is the method by which worms are used to turn organic materials (usually waste) into a humus-like substance known as Vermicast. The term vermicast is also termed as worm castings, worm manure, worm feces and worm humus. Vermicompost includes not only worm castings, but also bedding materials and organic waste in different phases of decomposition. It also includes worms that are at different stages of development and microorganisms involved in the composting process.

In the home garden, earthworm castings often contain between 5 and 11 times more nitrogen, phosphorous, and potassium than the surrounding soil.

Types of vermicomposting worms:

On the basis of their feeding habits, they are classified as detrivores and geophages. Detrivores feed on plant litter or dead roots, and other plant debris or on mammalian dung on or near the soil surface. These worms are referred to as humus formers and comprise the epigeic and anecic forms. Some examples of detrivorous worms are Perionyx *excavatus*, *Eisenia fetida*, *Eudrilus euginae*, *Lampito mauritii*, *Polypheretima elongata*, *Octochaetona serrata* and *Octochaetona curensis*. Geophagous worms, feed below the surface and intake greater quantities of organically rich soil. Red worm species in both *Eisenia fetida* and *Lumbricus rubellus* are composters, living naturally in soils that contain a lot of organic matter. For this reason, they are often used together, with *Eisenia fetida* on the surface and *Lumbricus rubellus* farther down, in vermicomposting systems (Soobhany *et al., 2015).*

Process of vermicomposting:

1. **Feeding materials:**

   Worms can eat dung from animals, agricultural waste, residues from vegetables, waste from the food market, waste from the flower market,

agro-industrial waste, waste from the fruit market and all other bio-degradable waste. Before being used for vermicompost production, cattle dung should be dried in open sunlight. Depending on the feedstock being used, temperature, moisture levels and the density of the worm population, the exact loading rate (at which raw feedstock will be applied to a worm bed) can differ. Proper loading rates require no inclusion of new feedstock until the bulk of the feedstock previously introduced has been decomposed. A high protein feedstock such as grains, mash, or cottonseed meal is added if worms are not growing.

2. Bedding materials

As bedding products, certain agricultural residues may be used, such as plant waste and solid composted manure. In general, because of bedding content's effect on increasing soil pH, which is harmful to worms, the bedding content should maintain moisture, stay loose and aerated, and be low in protein and nitrogen. The bedding content should be varied to provide the earthworms with a variety of nutrients and to create richer compost.

Suitable bedding materials includecoir waste, Cardboard, Shredded fall leaves, sawdust, chopped straw, mulched paper such as newspaper, semi-composted solid manure, By shredding raw materials into small pieces, decomposition can be accelerated.

3. Blending

To achieve a near optimal C/N ratio of 30:1-40:1, carbonaceous substances such as sawdust, paper and straw can be combined with nitrogen-rich products such as sewage sludge, biogas slurry, and fish scraps. Good quality compost, rich in main and micro nutrients, is produced by a varied mixture of substances.

4. Pre-composting/Half digestion:

In order to avoid worm systems from feeling so much sun, manure feedstocks and bedding should be pre-composted. When introduced into the worm systems, fresh manures produce a lot of energy that transfers into additional heat. Strong heat in the beds of worms can be deadly. The bedding and feeding materials are then combined, watered and allowed to ferment for approximately two to three weeks. It is necessary to hold the raw materials in piles to allow the temperature to exceed 50-55^{o} C.

The substance is overturned 3 to 4 times during this phase to get the temperature down and to aid in uniform decomposition. It is passed to the vermicompost production method as the material becomes very fragile, and worms are inserted into it ranging from a few days to a few weeks old.

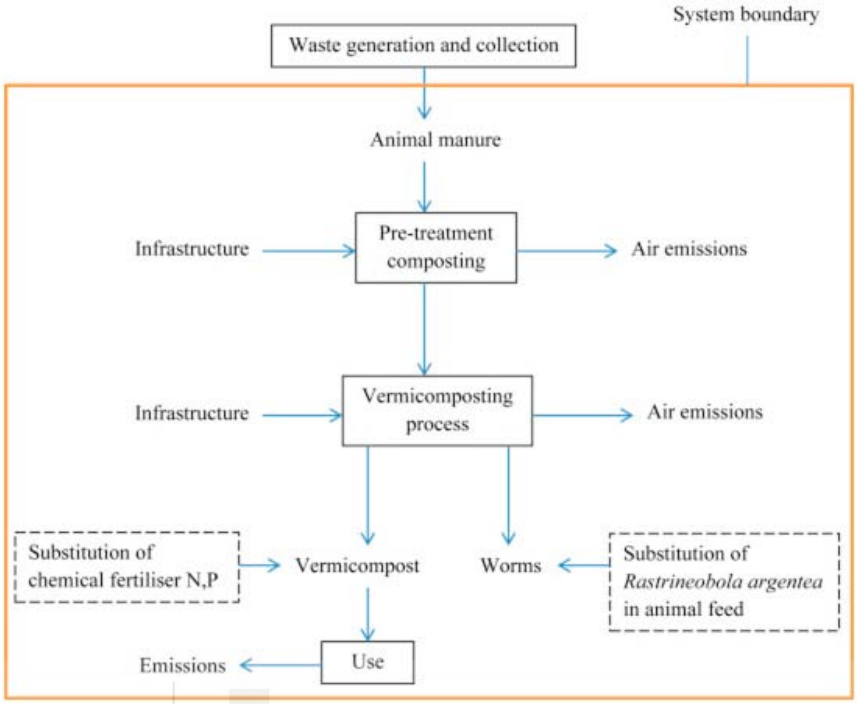


Figure 10: Process of Vermicomposting (Souce: Sahariah *et al, 2015)*

5. Moisture, temperature and pH:

50-60 percent is the optimal moisture level for maintaining aerobic conditions. The temperature should be within 25-30° C of the stacks. The activity of microflora and earthworms can be decreased by higher or lower temperatures. The height of bed can help to regulate the increase in temperature. The raw materials pH should not be greater than 6.5 to 7.

Methods of vermicomposting:

- A 1 meter by 1 meter by 0.3 meter container carries about 30-40 kg of bedding and feeding materials. It is possible to prepare a vermiculture bed or worm bed (3 cm) by putting dust or husk or coir waste or sugar cane garbage in the bottom of the tub/container.
- The culture bed can be spread with a sheet of fine sand (3 cm) followed by a layer of garden soil (3 cm). A15-20 cm sheet of organic waste material (pre-composted/half digested) can be spread on the worm bed.
- It is sprinkled with rock phosphate powder (to increase the content of phosphate) if required. Soil or cow dung is used to cover the organic layer with (sprinkle cow dung slurry).
- The selected earthworms are released through the cracks created (1000-1500). In order to prevent birds from eating the earthworms, cover the ring with wire mesh or gunny sack. Water is sprinkled to maintain adequate humidity and temperature regularly/daily. l.

Harvesting of vermicompost:

- In about 3 months, the vermicompost is ready (may vary depending on organic waste used as substrate). It will be black, granular, lightweight and humus-rich. Vermicompost harvesting requires manual isolation of

worms from the castings

- Watering is stopped for two to three days before emptying the beds to facilitate the removal of the worms from the compost. The worms will be pushed to the bottom of the bed by this. For new culture beds, the worms are collected. To retrieve the cocoons, young worms, and unconsumed organic waste, the gathered vermicompost is dried and passed through a 3 mm sieve. For seeding the new culture beds, cocoons and young worms are used.

Storage and packing of vermicompost:

- The harvested vermicompost should be stored in dark, cool location.
- It should have moisture of at least 40 percent.
- Sunlight should not fall on the content being composted.
- At the point of sale, packaging can be done.
- Periodic sprinkling of water can be done to retain the level of moisture and also to maintain a beneficial microbial population if it is kept in an open location.
- Vermicompost may be preserved for duration of one year without loss of quality if the moisture level is kept at 40%.

5.10 USE OF AGRO WASTE IN MUSHROOM CULTIVATION

Agricultural wastes are rich in lignocellulosic components which are difficult to breakdown, but can effectively be done mushroom cultivation. Mushrooms are fleshly fungi, spore bearing fruiting bodies which are produced above ground on soil. They often refer to fruiting body of the gill fungi, which do not contain chlorophyll like green plants and as a result cannot manufacture food by their own. They are very nutritious products that can be generated from lignocellulosic waste materials. The bioconversion of agricultural wastes into a value added products is a good mean of their use. The property of edible mushroom fungi to convert complex organic compounds into simpler one's is used to transform the useless agricultural waste into valuable product. Various edible mushroom strains are cultivated worldwide (Udayasimha L, Vijayalakshmi YC 2012). Some of them are given below:

- Button -*Agaricus*
- Oyster -*Pleurotous*
- Shiitake -*Lentinula Edodes*
- Straw -*Volvallella volvacea*
- Chinese mushroom - *Ganoderma*

Besides having many nutrional values they are also useful in waste management. The choice of species to cultivate depends on the availability of growth media. Oyster mushroom is the third most cultivated edible

mushroom in the world. Oyster mushrooms are easiest to grow as they can grow on many substrates but their cultivation has one drawback as some people are allergic to their spores. In these cases, air-cleaning equipment or respirators are necessary in order to safely work in the production facility. Because of the short shelf life this species offer a special advantage to the local grower who markets directly and can continuously deliver a fresh, high-quality product (Reis et al., 2012).

Two mycorrhizal mushrooms, morels and truffles are commercially cultivated. Mushroom cultivation offers benefits to market gardens when it is integrated into the existing production system. Mushrooms are rich in various nutrients such as:

- **Protein**- Protein content of dry weight is between 18% and 37%.
- **Fat-** Fat is present at low rate, content between 1-8%. The high content of linollic acids is one of the reasons why mushrooms are considered healthy food.
- **Vitamins and minerals -** Mushrooms are a good source of vitamins such as thiamine (Vitamin B), Riboflavin (vitamin B2) and ascorbic acid (vitamin C), folic acid. They also contain significant amounts of phosphorus, sodium, potassium, calcium, magnesium, iron and Zinc.

Protein content of mushroom in paddy was significantly higher than in wheat straw while lipid content of mushrooms was higher in wheat straw than paddy straw. Mushrooms have medicinal values as they contain substances which lower the cholesterol level in serum and liver which in turn makes it good for those suffering from heart diseases. Some of them contain substances, which suppress the growth rate of tumors (Reis et al., 2012)

Free radicals can damage body cells and induce cancers. Free radicals are the result of specific transformation process. Many bio active compounds protect the body against these radicals. These substances are often called anti-oxidants and are present in many mushrooms. In other words, the body immunity is boosted. This will be a relief to those suffering from HIV/AIDS (Reis et al., 2012).

Agricultural Wastes

Agricultural wastes are the good source for the cultivation of mushrooms. Some of them are most commonly used such as wheat straw, paddy straw, rice straw, rice bran, molasses, coffee straw, banana leaves, tea leaves, cotton straw, saw dust etc.

For the cultivation of *Pleurotus* rice straw, wheat straw and cotton straw are the substrates that are commonly used while for *Agaricus*, it is wheat straw which is usually used. A disadvantage of straw is that it should be prepared first, especially if mushrooms are to be grown indoors. Straw is laden with other microbes, and it is necessary to get rid of those tiny competitors, as there will be no scope of mushroom mycelium to grow in their presence.

Rice bran, coffee pulps are the main substrates used for the cultivation of *Lentinula edodes*. Banana leaves and tea leaves are used for *Volvallella* and *Pleurotus* respectively. Reis et al., (2012) proposed using banana leaves for

the cultivation of *Pleurotus spp*.

Ganoderma can be cultivated using sawdust. Sawdust itself is often not nutritious enough and needs to be supplemented with a nitrogen source such as bran, urea, sunflower seed and horse manure.

Cultivation of oyster mushroom is of most concern as its spores are allergic to some people, so related preventive measures should be done in working facility. Besides this, oyster mushrooms have a short life span, so they are beneficial to those growers who can sell them fresh in market (Tables 1-3).

Table 5.1: Various types of agricultural wastes used for mushroom cultivation. (Source: Nicolcioiu et al., 2016)

Sl no.	Agriculture waste	Strains
1	• Agricultural waste • .Rice straw • Wheat straw • Cotton straw • Tea leaves • Banana leaves	*Pleurotus sp.*
2.	• Wheat straw	*Agaricus bisporus*
3	• Rice bran • Coffee pulp	*Lentinula edodes*
4.	• Tea leaves	*Volvallella*
5.	• Sawdust	*Ganoderma*

Table 5.2: Composition of agricultural wastes used for mushroom cultivation. (Source: Nicolcioiu et al., 2016)

1.	Wheat straw	1% protein 13% lignin 39% hemicelluloses 40% cellulose
2.	Rice straw	41% cellulose 14% lignin 0.8% total nitrogen 0.25% P2O5 0.3% K2O 6% SiO2 pH 6.9
3.	Sugarcane bagasse	Cellulose 35-40% Hemicellulose 20-25% Lignin 18-24% Ash 1-4% Waxes <1% Nitrogen 0.7%

Table 5.3: Combination of substrates reported on various strains and their effect. (Source: Nicolcioiu et al., 2016)

S. No	Substrate Composition	strain	Effects
1.	Barley straw+wheat bran and wood chips+soybean powder+rice bran treatments	*Pleurotus eryngii*	4.64% protein content
2.	Wheat straw+wheat bran+soybean powder treatment	*Pleurotus eryngii*	13.66% protein content
3.	soybean straw+wheat straw	*Pleurotus sajorcaju*	87.3% Biological efficiency
4.	soybean straw+saw dust	*Pleurotus sajorcaju*	43.8% Biological efficiency
5.	corncob (CC)+sugarcane bagasse	*Pleurotus ostreatus*	corncob (CC)+sugarcane bagasse

Correlation of Agricultural Wastes Composition with Mushroom Cultivation

For high yield of mushroom cultivation, it is necessary that the entire nutritional requirement should be fulfilled in optimum concentration as various researches has reported low yield when nutrients in a medium are either in low or high concentration. Banana stalk and Bahia grass are used for the cultivation of *Pleurotus sajor-caju* with biological efficiency of 74.4% and 74.12% respectively but there is a low yield when they are supplemented with other components. This can be due to high nitrogen concentration which hinders its yield.

Growth of *Pleurotus ostreaus* resulted similar in paddy straw and wheat straw while in sugarcane bagasse it resulted in low yield. Reason behind this selective high yield must be appropriate concentration of lignin, hemicelluloses, cellulose in substrate.

There is a Positive correlation of cellulose: lignin with mycelia growth and high yield in *Pleurotus ostreatus* and carbon: nitrogen ratio with mushroom yield in *Pleurotus eryngii* and *Agaricus aegerita* while in *V. volvacea* strains high yield is related to cellulose content (Thongklang and Luangharn, 2016).

Combination of Agricultural Substrates Used For Cultivation

In addition to the use of supplements with agricultural wastes as a substrate, various combination of agricultural wastes are also used for the cultivation and are reported to be optimal substrate.

Vegetable waste when used in combination with paddy straw resulted in high yield of oyster mushroom. To cultivate *P. ostreatus* sawdust in addition to rice husks is reported as an optimal substrate. The quality of *P. eryngii* was significantly affected by substrate ingredients. On barley straw and sugar beet pulp substrate complemented with rice bran, highest mushroom fresh weight and moisture content were achieved.

For *Pleurotus sajor-caju*, combination of soybean straw, wheat straw showed significantly highest yield while soybean straw and saw dust combination showed significantly lesser yield.

Supplements Used With Agricultural Wastes

Agricultural wastes are used in addition to various supplements such as gypsum, lime and urea. Gypsum contributes as a calcium source and regulates the acidity level. Water holding capacity of gypsum is high which prevent excess wetting of the substrate. Lime is used to adjust pH. Mushroom cultivation needs appropriate nitrogen content for high yield, which can be fulfil by various components such as urea, bran, sunflower seed, molasses, horse manure.

Optimum Conditions for Cultivation

Besides having appropriate composition for significantly high yield of mushroom, optimum conditions of the environment during cultivation should also be maintained. Given below are the usual optimum conditions that should be maintained during cultivation.

- Temperatures of 15-35°C
- pH of about 6.5
- Carbon dioxide (CO2) level to be between 15-20%
- Humidity to be between 86-90%
- CO2 should be between 0-0.6percent.
- Temperatures and humidity levels should be regulated at 86% and 10-28°C respectively.

5.11 ADVANTAGES AND COST CONSIDERATION

India, being a second major producer of vegetables in the world; contributes 14% of total world vegetable production. Taking estimated production of fruits and vegetables in India at 150 million tons, the total waste generation comes to about 50 million tons per annum. Due to their chemical composition fruits and vegetables wastes are more prone to spoilage than cereals, which create unhygienic condition leading to spread of diseases and loss of resources. The vegetable wastes are a rich in nitrogen and carbohydrate but are not fit for consumption. These wastes can be utilized for the production of various types of mushroom such as the oyster variety.

In the recent times the waste management is of most concern. Proper management and execution of waste disposal practices have become today's need. The inappropriate management of waste gives rise to many problems such as rapid spread of infectious diseases, development of new varieties of diseases. The exponential increase in the present amount of waste produced brings to notice an immediate requirement of solution to overcome this problem.

An agricultural waste consists of lignin and cellulose, which are difficult to breakdown. They are insoluble and bind to inert substances in soil and get out of reach of bacterial culture present in soil. While mushroom's mycelium releases extracellular enzymes, which are responsible for the lignin degradation. *Pleurotus* and *Lentinus* have their own enzymes systems based on endoglucanase, laccase and phenoloxidases. The large amount of agricultural wastes and appropriate climatic conditions provide massive scope for oyster mushroom cultivation in Sagar, M.P.

An agricultural waste provides the opportunity for cost effective farming. Even after being used for mushroom cultivation, it can be used later on as manure for agricultural field as now the nutrient contents are at acceptable range. Cultivation of mushroom on these residual wastes is one of the most eco-friendly practices to fight the malnutrition and environmental pollution caused by these wastes. Various researches is still going on to exploit the potential of agricultural wastes either by using them in combination or by giving them pre-treatment.

5.12 PROCESS AND NEWLY EMERGING TECHNOLOGIES

Mushroom growing involves spawn production, composting, cultivation. Steps are shown in figure 11.

Figure 5.11: Three steps in mushroom cultivation (Source: Ralph and Kurtzman, 1994)

Cultivation technology is different for different mushrooms. Proper knowledge on mushroom life cycle and good training of all the steps is a must before starting cultivation of any mushroom. However the basic steps are the same for majority of mushrooms (figure11).

- The first step before starting cultivation is to procure or produce spawn of good quality.
- Second step is to prepare the substrate of good quality. Method of spawning, that is mixing of spawn in compost, and amount of spawn required will also vary in different mushrooms. In some cases spawn

may be mixed thoroughly whereas in other cases it may be put layer wise. Spawning in some cases can be done in open under hygienic conditions whereas in other cases, particularly where the substrate has been autoclaved, the spawning can be done only under sterile conditions. We need only half kg to one kg of spawn for 100 kg of compost in button mushroom, where as in oyster we need 2.5 kg and in milky mushroom we may require up to 5 kg spawn for 100 kg of substrate.

- The third step is cropping. After spawn run, that is allowing the fungus to spread throughout the substrate, we take steps to induce formation of mushrooms. In some cases it is required to put a layer of casing material whereas in other cases fruiting can be obtained as such. In all cases, to induce fruiting some sort of change is required. For example in case of button mushroom temperature is lowered from 25 to 17°C and carbon dioxide levels are lowered by giving fresh air. In Oyster, to induce fruiting both fresh air and diffused light is necessary.

In India, mushroom cultivation in rural areas has emerged as an important activity for educated, school dropouts, women, landless people, etc. Considering the demand for quality foods, mushroom cultivation has emerged as an important avocation. Many commercial units that grow mushrooms under controlled conditions have also been set up in different parts of our country. However, before taking up this venture a thorough knowledge of the subject and scientific aptitude towards agriculture is necessary.

Check Your Progress 2

Note: a) Use the space given below for your answers.

b) Check your answers with those given at the end of the unit

1. What is Composting?

……

……

……

……

……

2. What is Vermicomposting?

……

……

……

……

……

……

3. What is Mushroom cultivation?

…….

…….

…….

…….

…….

5.13 LET US SUM UP

We have studied the degradation of cellulose, hemicellulose, chitinn and lignin. We have studied the many aspects of composting, the assessment of the importance of vermicomposting and mushroom cultivation by utilizing agro residues. .

5.14 KEY WORDS

Biodegradation: Biodegradation is the degradation of the materials into environmentally acceptable products such as water, carbon dioxide, and biomass by the action of naturally available microorganisms under normal environmental conditions.

Cellulose: Cellulose, a complex carbohydrate, or polysaccharide, consisting of 3000 or more glucose units.

Lignin: Lignin is an important organic polymer which is abundant in cell walls of some specific cells. It has many biological functions such as water transport, mechanical support and resistance to various stresses.

Hemicelulose: Hemicellulose is a natural polymer like cellulose, consisting of carbohydrate monomers.

Chitin: A fibrous substance consisting of polysaccharides, which is the major constituent in the exoskeleton of arthropods and the cell walls of fungi.

Case Study:

Exploring the microbiota dynamics related to vegetable biomasses degradation and study of lignocellulose-degrading bacteria for industrial biotechnological application

The aims of this study were to evaluate the microbial diversity of different lignocellulosic biomasses during degradation under natural conditions and to isolate, select, characterise new well-adapted bacterial strains to detect potentially improved enzyme-producing bacteria. The microbiota of biomass piles of *Arundo donax*, Eucalyptus camaldulensis and *Populus nigra* were evaluated by high-throughput sequencing. A highly complex bacterial community was found, composed of ubiquitous bacteria, with the highest representation by the Actinobacteria, Proteobacteria, Bacteroidetes and

Firmicutes phyla. The abundances of the major and minor taxa retrieved during the process were determined by the selective pressure produced by the lignocellulosic plant species and degradation conditions. Moreover, cellulolytic bacteria were isolated using differential substrates and screened for cellulase, cellobiase, xylanase, pectinase and ligninase activities. Forty strains that showed multienzymatic activity were selected and identified. The highest endo-cellulase activity was seen in *Promicromonospora sukumoe* CE86 and *Isoptericola variabilis* CA84, which were able to degrade cellulose, cellobiose and xylan. Sixty-two percent of bacterial strains tested exhibited high extracellular endo-1,4-ß-glucanase activity in liquid media. These approaches show that the microbiota of lignocellulosic biomasses can be considered an important source of bacterial strains to upgrade the feasibility of lignocellulose conversion for the 'greener' technology of second-generation biofuels.

5.15 SUGGESTED FURTHER READING/ REFERENCES

- Scheller H. V and Ulvskov P. (2010). Hemicelluloses. Annual Review of Plant Biology, 61(1), 263–289. doi:10.1146/annurev-arplant-042809-112315
- Xiros C, Katapodis P, Christakopoulos P. Factors affecting cellulose and hemicellulose hydrolysis of alkali-treated brewers spent grain by Fusarium oxysporum enzyme extract. Bioresour Technol. 2011 Jan;102(2):1688-96. DOI: 10.1016/j.biortech.2010.09.108.
- López-Mondéjar R, Zühlke D, Becher D, Riedel K, Baldrian P. (2016). Cellulose and hemicellulose decomposition by forest soil bacteria proceeds by the action of structurally variable enzymatic systems. Sci Rep. Published 2016 Apr 29. doi:10.1038/srep25279
- Brunner, G. (2014). Processing of Biomass with Hydrothermal and Supercritical Water. Supercritical Fluid Science and Technology, 395–509.doi:10.1016/b978-0-444-59413-6.00008-x
- Gupta, R., Mehta, G., Deswal, D., Sharma, S., Jain, K. K., Kuhad, R. C., & Singh, A. (2013). Cellulases and Their Biotechnological Applications. Biotechnology for Environmental Management and Resource Recovery, 89–106.DOI:10.1007/978-81-322-0876-1_6
- Moussian B. (2019) Chitin: Structure, Chemistry and Biology. In: Yang Q., Fukamizo T. (eds) Targeting Chitin-containing Organisms. Advances in Experimental Medicine and Biology, vol 1142. Springer, Singapore. https://doi.org/10.1007/978-981-13-7318-3_2
- Casadidio C, Peregrina DV, Gigliobianco MR, Deng S, Censi R, Di Martino P. Chitin and Chitosans: Characteristics, Eco-Friendly Processes, and Applications in Cosmetic Science. Mar Drugs. 2019;17(6):369. Published 2019 Jun 21. doi:10.3390/md17060369

- Vinardell, Maria Pilar, and Montserrat Mitjans. "Lignins and Their Derivatives with Beneficial Effects on Human Health." International journal of molecular sciences 18,6 1219. 7 Jun. 2017, doi:10.3390/ijms18061219
- Chapter 2 – Structure and Characteristics of Lignin. Lignin Chemistry and Applications. Elsevier. 2019. Pages 25-50. https://doi.org/10.1016/B978-0-12-813941-7.00002-3.
- Udayasimha L, Vijayalakshmi YC (2012) Sustainable Waste Management By Growing Mushroom (Pleurotusflorida) On Anaerobically Digested Waste And Agro Residues. Int J Eng Res Tech 1: 12.
- Nicolcioiu MB, Popa G, Matei F (2016) Mushroom Mycelia Cultivation on Different Agricultural Waste Substrates. Romania Scientific Bulletin Series F. Biotechnologies Vol. Xx.
- Thongklang N, Luangharn T (2016) Testing agricultural wastes for the production of Pleurotus ostreatus. Mycosphere 7: 766-772.
- Ralph H, Kurtzman J (1994) Nutritional needs of mushroom and substrate supplements. In: Nair MC (editor), Advances in mushroom biotechnology: 106-110.
- Rani P, Kalyani N, Prathiba K (2008) Evaluation of Lignocellulosic Wastes for Production of Edible Mushrooms. Applied Biochemistry And Biotechnology 151: 151-159.
- Reis FS, Barros L, Martins A, Ferreira I (2012) Chemical Composition And Nutritional Value Of The Most Widely Appreciated Cultivated Mushrooms: An Inter-Species Comparative Study. Food and Chemical Toxicology 50: 191-197.
- Vyas D, Chaubey A, Dehariya P, Wagay AJ, Bajpai A, at al. (2009) Perspectives on Agripreneurship And Rural Development, ICARD. International Conference On Agripreneurship & Rural Development, BHU, Varanasi, India: 463-470.
- Kumar S., 2011. Review Article: Composting of municipal solid waste. Critical Reviews in Biotechnology. 31(2), 112-136.
- Ipek, U., Obek, E., Akca, L., Arslan, E.I., Hasar, H., Dogru, M., Baykara, O., 2002. Determination of degradation of radioactivity and its kinetics in aerobic composting. Bioresource Technology 84(3), 283-286.
- N. Soobhany *et al.* Comparative assessment of heavy metal content during the composting and vermicomposting of municipal solid waste employing *Eudrilus eugeniae*Waste Manag. (2015).
- B. Sahariah *et al.* Efficacy of bioconversion of paper mill bamboo sludge and lime waste by composting and vermiconversion technologies Chemosphere (2014).

5.16 ANSWERS TO CHECK YOUR PROGRESS

Check Your Progress 1

1. Biodegradation is biological catalyzed reduction in complexity of chemical compounds. It is the process by which organic substances are broken down into smaller compounds by living microbial organisms. When biodegradation is complete, the process is called "mineralization". However, in most cases the term biodegradation is generally used to describe almost any biologically mediated change in a substrate.

2. Cellulose degradation occurs in three simple steps;

1. Hydrolysis by endoglucanases

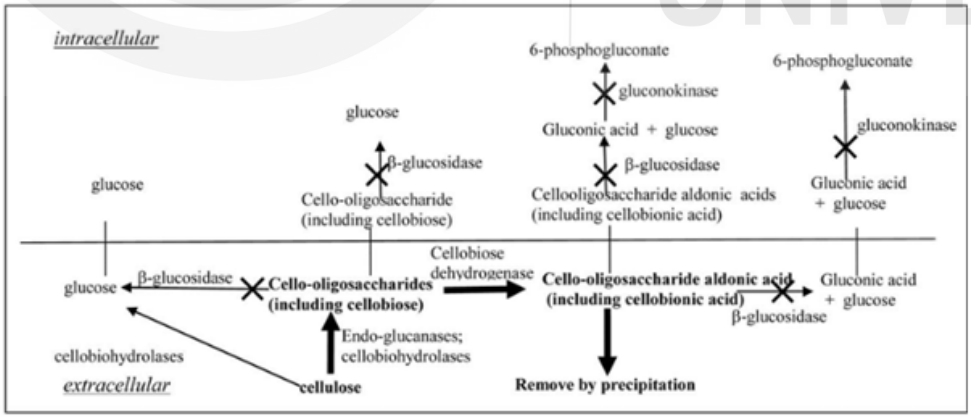
The first step in the degradation of cellulose is the action of endoglucanases that randomly attack the cellulose fibrils. This step results in a decrease in the size of cellulose chains as it degrades the polymer into smaller fragments. The enzyme acts internally at random points of the polymer.

2. Hydrolysis by exoglucanases

Exoglucanases act on the smaller fragments resulting in even smaller units of tetrasaccharides or disaccharides. Exoglucanases act on the reducing end of the fragments to form either dimeric units or cellobiose.

3. Hydrolysis by β-glucosidase

β-glucosidase or cellobiose act on the dimeric units of glucose of cellobiose to form monomeric units, glucose. This is the final step of cellulose degradation that results in the formation of free individual units of the glucose molecule.



Simple Steps of cellulose degradation

3. Chitin is a complex homopolysaccharide consisting of units of amino sugar glucosamine that accounts for the second most abundant polysaccharide of nature after cellulose.

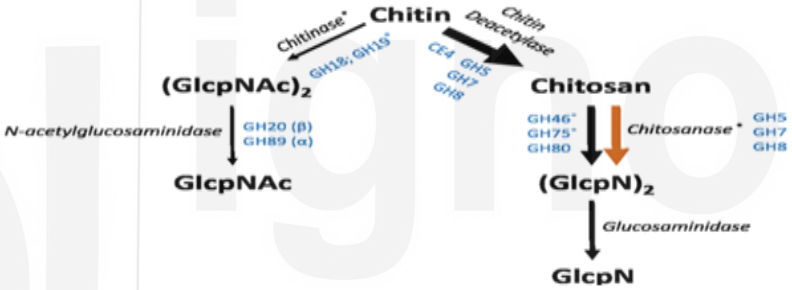
Microbial chitin degradation occurs by one of the two mechanisms; chitinoclastic mechanism and deacetylation mechanism.

1. Chitinoclastic

In this mechanism, the substrate is acted upon by the chitinolytic system, consisting of chitinases. Exochitinase breakdown acetylchitobiose units from the non-reducing end of the polysaccharide chain. Endochitinase cleaves glycosidic linkages randomly along the chain, eventually resulting in the formation of diacetylchitibiose as the major product, along with some tri-acetyl chitotriose.

2. Deacetylation

The group of enzymes involved in the deacetylation mechanism is termed deacetylases. These enzymes catalyze the process of deacetylation of N-acetylglucosamine polymer. The hydrolysis of chitosan occurs in the presence of chitosanases that breakdown the linkages between the β-glucosamine units linked together by β-1,4-glycosidic linkages. This cleavage results in the release of chitobiose (glucosaminyl-(1-4)- β-glucosaminide) which is then



Check Your Progress 2

1. Composting is the natural process of turning organic matter in waste into a beneficial fertilizer that can benefit both soil and plants. Composting converts organic waste such as food waste, manure, leaves, grass trimmings, paper, wood, feathers, agricultural residue, etc. into beneficial organic fertilizer by using various microorganisms such as bacteria and fungus.
2. The term vermiculture refers to the cultivation or production of earthworms. Vermicomposting is the method by which worms are used to turn organic materials (usually waste) into a humus-like substance known as Vermicast. The term vermicast is also termed as worm castings, worm manure, worm feces and worm humus. Vermicompost includes not only worm castings, but also bedding materials and organic waste in different phases of decomposition. It also includes worms that are at different stages of development and microorganisms involved in the composting process.
3. Mushroom growing involves spawn production, composting, cultivation.

Three steps in mushroom cultivation

Cultivation technology is different for different mushrooms. Proper knowledge on mushroom life cycle and good training of all the steps is a must before starting cultivation of any mushroom. However the basic steps are the same for majority of mushrooms (Fig. 1.10).

- The first step before starting cultivation is to procure or produce spawn of good quality.
- Second step is to prepare the substrate of good quality. Method of spawning, that is mixing of spawn in compost, and amount of spawn required will also vary in different mushrooms. In some cases spawn may be mixed thoroughly whereas in other cases it may be put layer wise. Spawning in some cases can be done in open under hygienic conditions whereas in other cases, particularly where the substrate has been autoclaved, the spawning can be done only under sterile conditions. We need only half kg to one kg of spawn for 100 kg of compost in button mushroom, where as in oyster we need 2.5 kg and in milky mushroom we may require up to 5 kg spawn for 100 kg of substrate.
- The third step is cropping. After spawn run, that is allowing the fungus to spread throughout the substrate, we take steps to induce formation of mushrooms. In some cases it is required to put a layer of casing material whereas in other cases fruiting can be obtained as such. In all cases, to induce fruiting some sort of change is required. For example in case of button mushroom temperature is lowered from 25 to 17°C and carbon dioxide levels are lowered by giving fresh air. In Oyster, to induce fruiting both fresh air and diffused light is necessary.

  In India, mushroom cultivation in rural areas has emerged as an important activity for educated, school dropouts, women, landless people, etc. Considering the demand for quality foods, mushroom cultivation has emerged as an important avocation. Many commercial units that grow mushrooms under controlled conditions have also been set up in different parts of our country. However, before taking up this venture a thorough knowledge of the subject and scientific aptitude towards agriculture is necessary.