

Carbon Cycle

Carbon Cycle

- The **carbon cycle** is the process that moves carbon between plants, animals, and microbes; minerals in the earth; and the atmosphere.
- Carbon is the fourth most abundant element in the universe. With its ability to form complex molecules such as DNA and proteins, carbon makes life on Earth possible.
- Carbon in the form of carbon dioxide (CO_2) is also an important part of our atmosphere, where it helps to control the Earth's temperature.
- Because only a tiny number of atoms reach the Earth from space, our planet is called a closed system.
- This means the Earth does not gain or lose carbon. But carbon does move constantly.
- Most carbon on Earth is stored in rocks and sediments.
- The rest is in the ocean, atmosphere, and in living organisms.
- Scientists use the term “carbon sinks” to refer to places where carbon is stored away from the atmosphere.

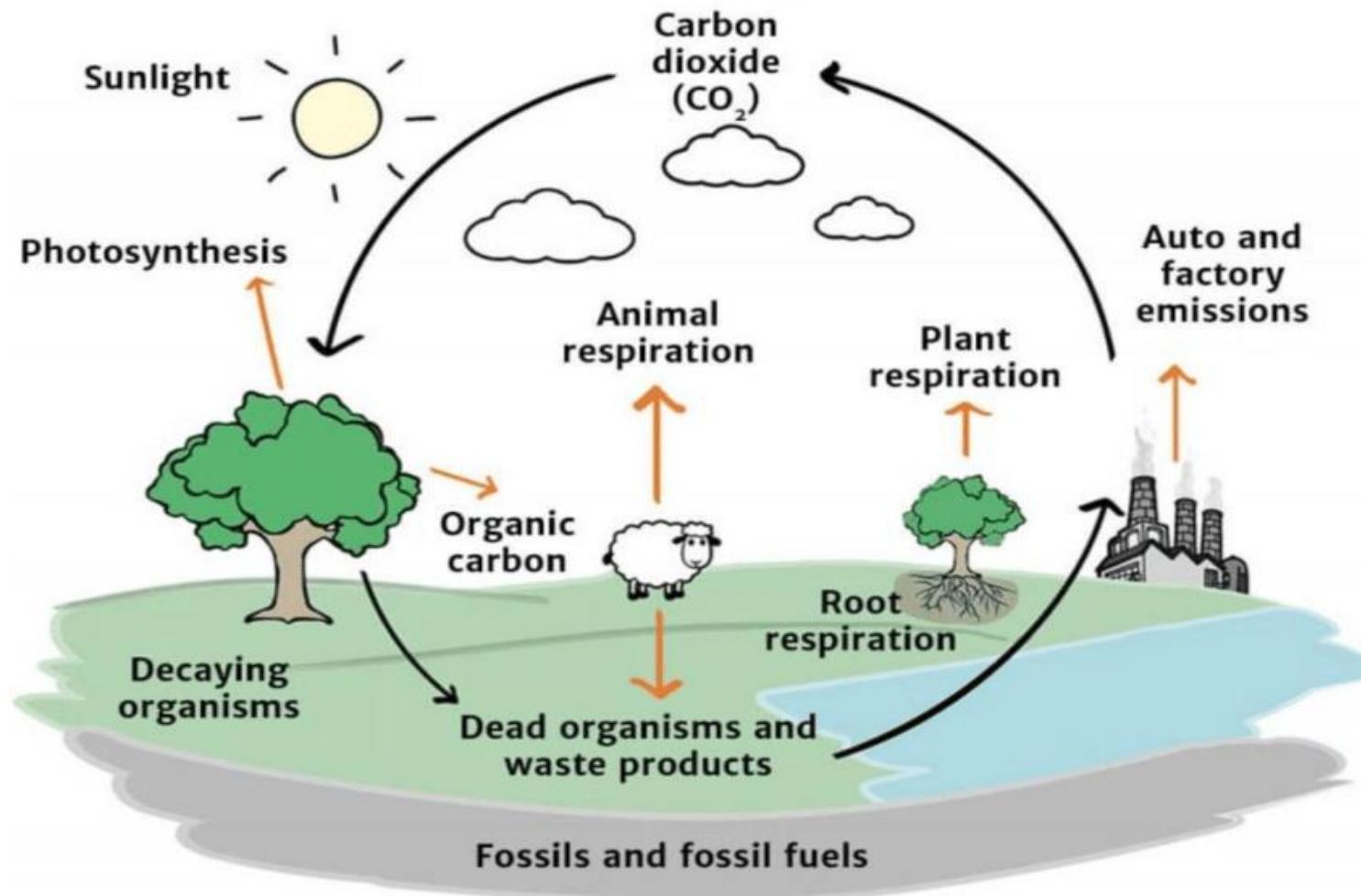
• Carbon Cycle

- Plants constantly exchange carbon with the atmosphere.
- Plants absorb carbon dioxide during photosynthesis and much of this carbon dioxide is then stored in roots, permafrost, grasslands, and forests.
- Plants and the [soil](#) then release carbon dioxide when they decay. Other organisms also release carbon dioxide as they live and die.
- For example, animals exhale carbon dioxide when they breathe and release carbon dioxide when they decompose.
- The oceans also exchange carbon with the atmosphere by absorbing carbon, which then sinks as it cools.
- In addition, carbon is stored in rocks and other geological deposits.
- For example, coal and other fossil fuels are made of carbon from plants that has been stored under the Earth's surface for millions of years.

• Carbon Cycle

- Humans have a huge effect on the carbon cycle when we burn wood, fossil fuels (such as oil, coal, and natural gas), and other forms of carbon.
- This action releases the stored carbon into the atmosphere, where it becomes a greenhouse gas.
- Greenhouse gases are gases in the atmosphere that absorb and release heat.
- Where carbon is in the cycle shapes our climate. As a greenhouse gas, carbon dioxide in the atmosphere helps to determine how warm the Earth is.
- Too little carbon dioxide and other greenhouse gases and the Earth would be frozen.
- Too much would turn the atmosphere into a furnace.
- That's why understanding the carbon cycle—and our role in that cycle—is critical to the Earth's future.

Carbon Cycle



Role Of Microorganism In Carbon Cycling

- **Soil Organic Carbon**
- Small (fresh) plant remains and tiny live soil organisms, decomposing (active) organic matter, and stable organic matter (humus) are the three main components that make up soil organic carbon.
- Root exudates, living and dead microorganisms, plant and animal waste, and soil biota all contribute to the decomposition of organic carbon (OC) in the soil.
- It serves as soil microbes' primary energy source.
- Heterotrophic microflora and microfauna, which include bacteria, fungi, actinomycetes, and protozoa, are responsible for decomposition.
- In addition to microflora and microfauna, numerous mesofauna species, including earthworms, are crucial to the early decomposition of organic waste.
- For the microflora, the breakdown of organic matter provides three benefits: (i) energy for growth, (ii) carbon for cell material production, and (iii) additional nutrients and elements required for cell growth.

Organic matter decomposition

- Almost all organic matter in soil is directly and indirectly derived from plants via photosynthesis.
- Plant and animal detritus and root exudates represent essential sources of energy and nutrients for soil microbial and faunal communities.
- Organic matter decomposition serves three functions for the micro flora:
 - (i) providing energy for growth,
 - (ii) supplying carbon for the formation of cell material, and
 - (iii) providing other nutrient, elements needed for cell growth.
- Soluble substances such as sugars and low molecular weight phenolic compounds are assimilated by the microorganisms and metabolized.
- However, a large fraction of plant litter is composed of high-molecular weight polymers such as celluloses, lignin and suberin, which cannot be directly assimilated by microorganisms.
- Rather, extracellular enzymes must first break them down.
- Organic residues added to the soil are first broken down into their basic components by extracellular enzymes; and the basic components are subsequently utilized by intracellular enzymes.

...Organic matter decomposition

- Sugars, water-soluble nitrogenous compounds, amino acids, lipids, starches and some of the hemicelluloses' are decomposed first at rapid rate, while insoluble compounds such as cellulose, hemicelluloses, lignin, proteins etc. which forms the major portion of organic matter are decomposed later slowly.
- Chitin is the **second most abundant natural biopolymer after cellulose** and occurs widely in arthropods, fungi, and marine organisms.
- In aquatic systems alone, **more than 10^{11} metric tons of chitin are produced annually**, representing an enormous pool of organic carbon.
- Without degradation, this carbon would remain locked in insoluble biomass.
- Chitin degradation is a **critical process in biogeochemical cycling** across terrestrial and aquatic ecosystems.

Plant Cell Wall

- The cell wall of a higher plant is made up of about 90% carbohydrates and 10% proteins.
- The main carbohydrate constituent is **cellulose**.
- About 36 cellulose chains are associated by interchain hydrogen bonds to a crystalline lattice structure known as a **microfibril**.
- The microfibrils have an unusually high tensile strength, are very resistant to chemical and biological degradations.
- However, many bacteria and fungi have cellulose-hydrolyzing enzymes.
- Hemicelluloses are also important constituents of the cell wall.
- Hemicelluloses consist of a variety of polysaccharides that contain, in addition to D-glucose, other carbohydrates such as the hexoses D-mannose, D-galactose, D-fucose, and the pentoses D-xylose and L-arabinose.
- Another major constituent is pectin, a mixture of polymers from sugar acids, such as D-galacturonic acid, which are connected by β -1,4 glycosidic links.
- Pectin makes the wall elastic and, together with the glycoproteins and the hemicellulose, forms the matrix in which the cellulose microfibrils are embedded.

... Plant Cell Wall

- When the cell has reached its final size and shape, another layer, the secondary wall, which consists mainly of cellulose, is added to the primary wall.
- The microfibrils in the sec. wall are arranged in a layered structure like plywood.
- The incorporation of lignin in the secondary wall causes the lignification of plant parts and the corresponding cells die, leaving the dead cells with only a supporting function (e.g., forming the branches and twigs of trees or the stems of herbaceous plants).
- Lignin is formed by the polymerization of the phenylpropane derivatives cumaryl alcohol, coniferyl alcohol, and sinapyl alcohol, resulting in a very solid structure.

Cellulose decomposition

- When cellulose is associated with pentosans (eg. xylans & mannans) it undergoes rapid decomposition, but when associated with lignin, the rate of decomposition is very slow.
- The decomposition of cellulose occurs in two stages:
 - (i) in the first stage the long chain of cellulase is broken down into cellobiose and then into glucose by the process of hydrolysis in the presence of enzymes cellulase and cellobiase, and
 - (ii) in second stage glucose is oxidized and converted CO_2 and water.
- In order to access this glucose for catabolism, the cellulose must be decomposed by extracellular enzymes.
- Cellulases have different specificities to hydrolyse the β -1,4-glycosidic linkages bonds that connect glucose units in the cellulose fiber.
- They are divided into three major classes:
 - endoglucanases (endo-1-4- β -glucanase) : internally cleave β -1,4-glycosidic bonds in the amorphous regions of cellulose thereby releasing reducing and nonreducing chain ends.

... Cellulose decomposition

- Exoglucanases : remove dimers (cellobiose) from the end of the cellulose chain.
- β -glucosidase : hydrolyse glucose dimers and in some cases gluco-oligosaccharides to glucose.
- These pieces are then transported into the cell for energy generation (catabolism) or production of biomass (anabolism).
- Fungi such as *Penicillium* and *Aspergillus*, and bacteria such as *Streptomyces* and *Pseudomonas* are important participants in the extracellular cleavage of cellulose.
- The most extensively studied sources of cellulolytic enzymes have been the fungi *Trichoderma* and *Phanerochaete* and the bacteria *Cellulomonas* (an aerobe) and *Clostridium thermocellum* (an anaerobe).

Hemicellulose decomposition

- Hemicelluloses are water-soluble polysaccharides and consist of hexoses, pentose, and uronic acids and are the major plant constituents second only in quantity of cellulose, and sources of energy and nutrients for soil micro flora.
- The hydrolysis is brought about by number of hemicellulolytic enzymes known as "hemicellulases" excreted by the microorganisms.
- On hydrolysis hemicelluloses are converted into soluble monosaccharide/sugars (e.g. xylose, arabinose, galactose and mannose) which are further converted to organic acids, alcohols, CO_2 and H_2O and uronic acids are broken down to pentose and CO_2 .
- Various microorganisms including fungi, bacteria and actinomycetes both aerobic and anaerobic are involved in the decomposition of hemicelluloses.

... Hemicellulose decomposition

- Hemicellulose hydrolysis demands cooperative action of several types of enzymes working at different levels of the hemicelluloytic matrix.
- This synergistic activity is necessary not only because of hemicellulose complexity but also because of its connection with the other plant cell wall components.
- According to their action on distinct substrates two types of enzymes are predominantly involved in hemicellulose degradation:
 - endo-1,4- β -xylanase : hydrolyse β -1,4-xylan chains, and generate xylo-oligosaccharides
 - exo-1,4- β -xylosidase : cleave xylobiose and xylo-oligosaccharides releasing xylose
- Mannan, as the major component of hemicellulose in softwood, is comprised of mannose residues or a combination of mannose and glucose residues also known as glucomannan.

... Hemicellulose decomposition

- β -Mannanases are endohydrolases that hydrolyse mannan fibers by cleaving β -1,4 bonds and producing new reducing and nonreducing ends.
- The hydrolytic action of β -mannanases on mannan is supported with β -mannosidase enzymes that carry out hydrolysis of terminal, nonreducing β -D-mannose residues.
- In case of glucomannan degradation, β -glucosidases can cleave the bond between one mannose and one glucose residue.
- One should be aware that the action of these enzymes strongly depends on the number and pattern formed by the substituted galactoses and other substitutions and on the action of other enzymes

Lignin

- Lignin is the most common aromatic organic compound found in the lignocellulose component of the plant cell wall.
- Lignin is the most abundant source of carbon in soil after cellulose.
- Its characteristic ability to absorb UV (ultraviolet) radiation makes it susceptible to degradation on being exposed to sunlight.
- The source of lignin in soil can be of plant origin or lignocellulosic waste from the food processing industry.
- Lignin is an amorphic three-dimensional polymer composed of phenylpropanoid subunits.
- It acts as a binding material and is involved in cross-linking of cellulose that provides extra strength, rigidity, and stiffness to the cell wall.
- Lignin protects plant cells from enzymatic hydrolysis and various other environmental stress conditions.
- The complex structure of lignin makes it recalcitrant to most degradation methods..

Lignin Structure and Its Biosynthesis

- In the plant cell, lignin is biosynthesized by the combination of three basic hydroxycinnamoyl alcohol monomers or monolignols:
 1. p-Coumaryl alcohol
 2. Coniferyl alcohol
 3. Sinapyl alcohol.
- These monolignols are often referred to as phenylpropanoids, which differ in the substitutions at the 3-C and 5-C positions in the aromatic ring.
- The natural polymerization of monolignols (lignification) starts with the oxidative formation of phenoxy radicals catalyzed by peroxidases and/or laccases.
- It is followed by combinatorial radical coupling, generating carbon–carbon and carbon–oxygen (ether) bonds.
- The emerging crosslinked network and aromatic nature of lignin explain its recalcitrance towards degradation.

Lignin degradation processes

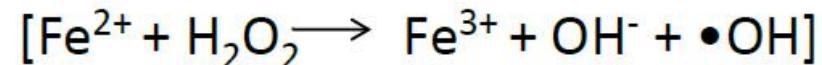
- The lignocellulosic complex in the plant cell wall contains approximately 40 to 60% cellulose, 20 to 40% hemicellulose, and 10 to 25% lignin.
- Certain enzymes from specialized bacteria and fungi have been identified by researchers that can catalyze a number of oxidative and hydroxylation reactions, depolymerize the phenolic and non-phenolic lignin polymer, and also mineralize the insoluble lignin.
- The biodegradation of lignocellulosic biomass has been widely studied in wood rotting Basidiomycetes microorganisms.
- These basidiomycetes are categorized as white-rot and brown-rot fungi.
- White-rot fungi are the most effective bio-degraders of lignocellulosic biomass (e.g., *Phanerochaete chrysosporium*, *Pleurotus ostreatus* and *Ceriporiopsis subvermispora*) and can degrade lignin faster than other microorganisms.

White-rot fungi

- White-rot fungi produce a number of extracellular enzymes that directly attack lignin, cellulose, and hemicellulose of the plant cell wall to decompose it.
- These enzymes include laccases and peroxidases, such as lignin peroxidase (LiP), manganese peroxidase (MnP), and versatile peroxidase (VP).
- **Laccases** catalyze the oxidation of polyphenols and methoxy-substituted phenols by generating free radicals.
- **Lignin peroxidases** catalyze the oxidative depolymerization of lignin with H_2O_2 acting as the oxidizing agent.
- These enzymes are relatively nonspecific, and can therefore oxidize phenolic aromatic substrates as well as various non-phenolic lignin model compounds.
- **Manganese peroxidases** use H_2O_2 to oxidize Mn^{2+} , which is present in wood and soils, thus generating reactive Mn^{3+} ions, mediates oxidation of a large number of phenolic substrates.
- **Versatile peroxidases** combine the properties of lignin peroxidases and manganese peroxidases, conferring the catalytic versatility.
- They can oxidize Mn^{2+} to Mn^{3+} like manganese peroxidases, but can also oxidize non-phenolic compounds in the same manner as lignin peroxidases.

Brown rot fungi

- Typical examples of brown-rot fungi include *Gloeophyllum trabeum*, *Postia placenta* and *Fomitopsis palustris*.
- The brown-rot fungi are less efficient in degrading lignin compared to white-rot fungi.
- Lignin degradation by brown-rot fungi mainly involves non-enzymatic oxidation reactions producing hydroxyl radicals via Fenton chemistry.



- Brown-rot fungi partially oxidize lignin via aromatic ring demethylation.
- They do not degrade lignin directly, but rather modify it by partial oxidization and then preferentially degrade the polysaccharides.
- Wood decomposed by brown-rot fungi is therefore characterized by a brown color arising from the residual lignin.
- In addition to color changes, wood decay by brown-rot fungi shrinks, breaks into brick-shaped pieces and crumbles to a brown powder.

Bacterial lignin degradation

- Research in terms of lignin degradation by bacteria has been limited.
- Many soil bacteria such as Actinomycetes are also capable of mineralizing and solubilizing polymeric lignin and lignin-related compounds.
- Bacteria (e.g., *Streptomyces viridosporus*, *Brucella*, *Ochrobactrum*, *Sphingobium*, *Sphingomonas*, *Pseudomonas putida* and *P. fluorescens*) could oxidize phenolics but not the non-phenolic compounds.
- Various types of cleavages in lignin molecules, e.g., aromatic ring, demethylation, and oxidation, are catalyzed by bacterial enzymes.
- Bacteria are relatively rich in another type of peroxidase, the so-called dye-decolorizing peroxidases (DyP).
- DyPs are mainly active at acidic pH and show a very broad substrate profile, including several classes of synthetic dyes, monophenolic compounds, veratryl alcohol, β -carotenes, Mn^{+2} and lignin model compounds.
- Laccases are ubiquitous in nature, found in plants, fungi, bacteria and insects.
- They are often secreted as extracellular catalysts and typically perform polymerization or depolymerization reactions.
- Similar to DyPs, the most studied bacterial laccases in lignin degradation are from actinomycetes, particularly from *Streptomyces* species.

The process of catabolic lignin degradation

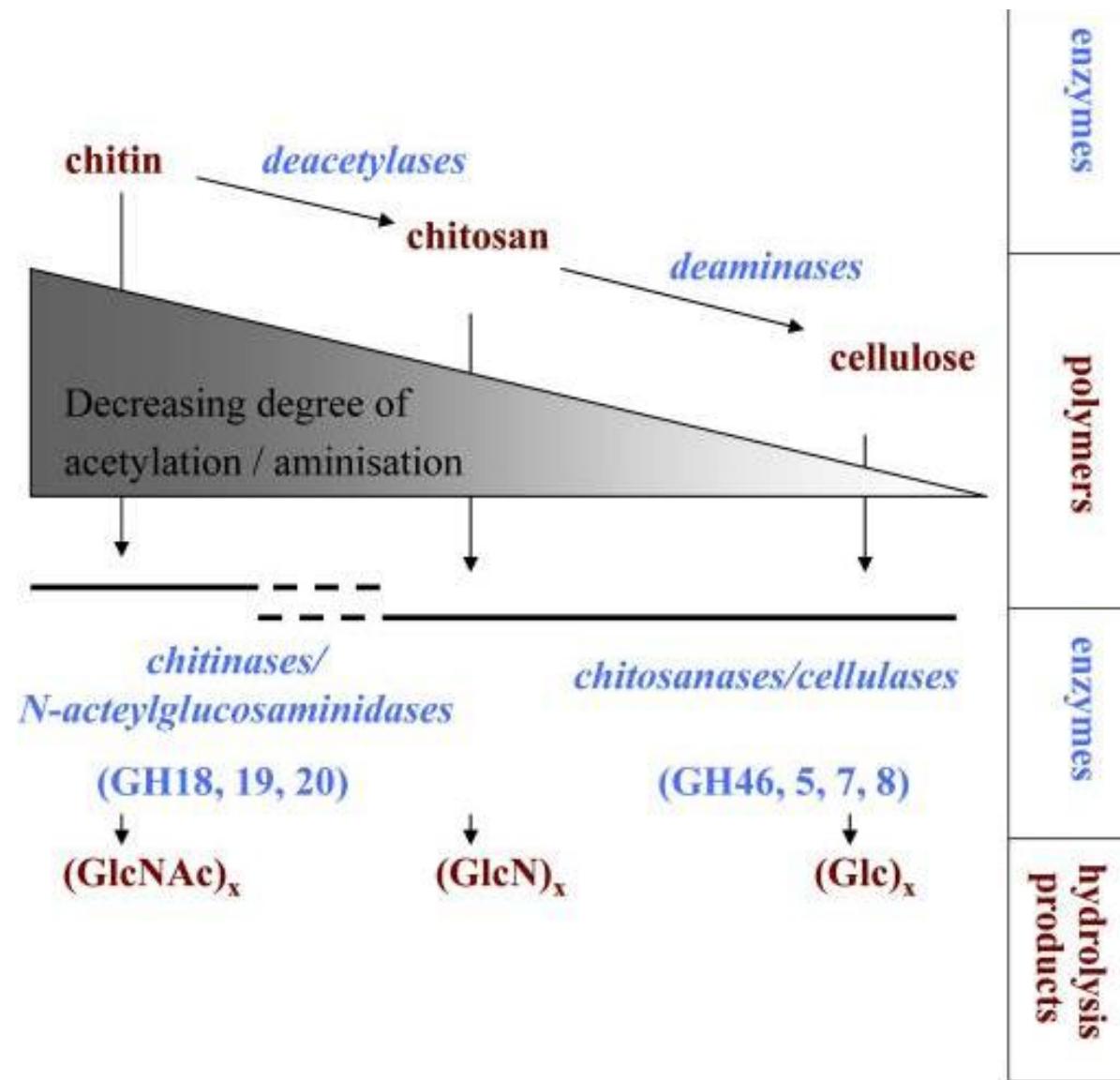
- The process involves:
 - cleavage of ether bonds between monomers
 - oxidative cleavage of the propane side chain
 - demethylation
 - benzene ring cleavage to keto adipic acid which is fed into the tricarboxylic acid cycle as a fatty acid.
- Most research has been concentrated on white-rot basidiomycete fungi, such as *Phanerochaete chrysosporium* (= *Sporotrichum pulverulentum*), which is able to mineralise lignin completely to carbon dioxide and water.
- The lignin-degradative system of *P. chrysosporium* appears after cessation of primary growth (that is, it is an aspect of the secondary metabolism of the organism) and can be induced by nitrogen starvation.

Chitin

- Chitin is one the most abundant polymers in nature and interacts with both carbon and nitrogen cycles.
- The occurrence of chitin is widespread in nature and chitin serves as a structural element in many organisms, e.g., fungi, crustaceans, insects or algae.
- Chitin is a $\beta(1,4)$ -linked homopolymer of N-acetyl glucosamine derivative of glucose, and it shares close structural similarity to cellulose.
- Chitin is classified into three different crystalline forms: the α -, β -, and γ -form, which differ in the orientation of chitin micro-fibrils.
 - In α -chitin, the chitin fibers are antiparallel, resulting in an orthorhombic orientation.
 - In β -chitin, the chitin fibers are parallel to each other, stabilized only by the intrasheet hydrogen bonding.
 - The γ -chitin conformation is characterized by alternating parallel and antiparallel aligned chitin fibers.

Chitin Biodegradation

- Chitinoclastic: Chitin degradation involves the initial hydrolysis of the $(1 \rightarrow 4)$ - β -glycoside bond, as seen for chitinase-catalyzed chitin degradation.
 - Chitinases are broadly divided into two groups; exo-chitinases and endo-chitinases.
 - Endochitinases split the polymeric chains at random sites internally, thereby forming the dimeric units of di-cetylchitobiose and soluble low molecular mass multimers of glucosamine as chitotriose and chitotetraose.
 - Exochitinases, in turn, are further divided into two groups; chitobiosidases and $1,4$ - β -glucosaminidases.
 - Chitobiosidases catalyze the subsequent release of di-acetylchitobiose beginning at the non-reducing of the chitin fibrils.
 - $1,4$ - β -glucosaminidases cleave the oligomeric products of endochitinase and chitobiosidases to produce monomeric products of glucosamine.
- Growth on chitin is not necessarily accompanied by the direct dissolution of its polymeric structure.
- **Deacetylation and Deamination:** Alternatively, chitin can be deacetylated to chitosan or possibly even cellulose-like forms, if it is further subjected to deamination (Figure 1).
- Chitinases and chitosanases overlap in substrate specificity, while their respective efficiency is controlled by the degree of deacetylation of the polymeric substrate (Figure 1).
- Besides specific chitosanases, also cellulases can possess considerable chitosan-cleaving activity.



Microorganisms involved in chitin degradation

- Bacterial species of *Vibrio*, *Photobacterium*, *Aeromonas*, *Cytophaga*, *Streptomyces*, *Photobacterium*, *Bacillus*, *Clostridium*, and *Chromobacterium* are well-known chitinolytic bacteria.
- Chitinolytic bacteria are also abundant in freshwaters, characteristic genera of *Serratia*, *Chromobacterium*, *Pseudomonas*, *Flavobacterium*, and *Bacillus*, with *Cytophaga johnsonae*.
- The primary habitat of chitinolytic fungi is the soil where the chitinolytic activity of fungi might even exceed that of bacteria.
- 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*.
- The chitinolytic system in these fungi is inducible, and the activity increases with the increase in the chitin-rich substrate.
- Myxomycetes (true slime molds) like *Physarum polycephalum* are a rich source of lytic enzymes that produce a complex of extracellular chitinases.

Factors affecting chitin degradation

- The process of chitin degradation occurs rapidly in the presence of free water and complete saturation.
- The addition of glucose in the media or soil decreases the rate of chitin degradation as the organisms tend to utilize the readily available source rather than chitin.
- Since most of the chitinolytic microorganisms are aerobic and thrive in high-oxygen environments, the rate of chitin degradation also increases.
- The presence of organic matter rich in chitin also increases the rate of chitin degradation.
- The increase in organic matter increases the substrate concentration. The rate of degradation might be slow at first as the microorganisms utilize more readily available energy forms, followed by chitin degradation.