Enzyme Science and Technology
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Module - XII
Enzyme Applications (Part-II)
Lecture - 47
Enzymes in Environmental Field

Hello everyone, this is Dr. Vishal Trivedi from Department of Biosciences and Bioengineering IIT, Guwahati. And what we were discussing, we were discussing about the different properties of the enzyme in the course Enzyme Science and Technology. And in this current module, we are discussing about the application of enzyme into the different fields which are required for the human welfare.

So, what we have discussed? We have discussed that the enzymes are required for the development of vaccines, enzymes are required for development of transgenic animals, medicines, genetically modified organisms, enzymes are also playing very crucial role in developing the different types of products related to the agriculture and as well as the basic culture.

And in this particular course, what we are discussing? We are discussing about the implication of enzyme into the food industry, medical field, genetic engineering and environment. And if you recall, in the previous module, we have discussed about the role of enzyme into the food industry and as well as the medical field.

And in this particular module, in our previous lecture, we have discussed about the role of enzyme into the medical field, how you can be able to utilize these enzyme for the development of different types of drugs for modulating the enzyme activity so that you can be able to control the pathophysiology and other kinds of symptomatic effects.

And you can also be able to treat the drugs right, treat the disease. Apart from that, we have also seen that how the different types of enzymes have the significant role in drug metabolism and detoxifications.

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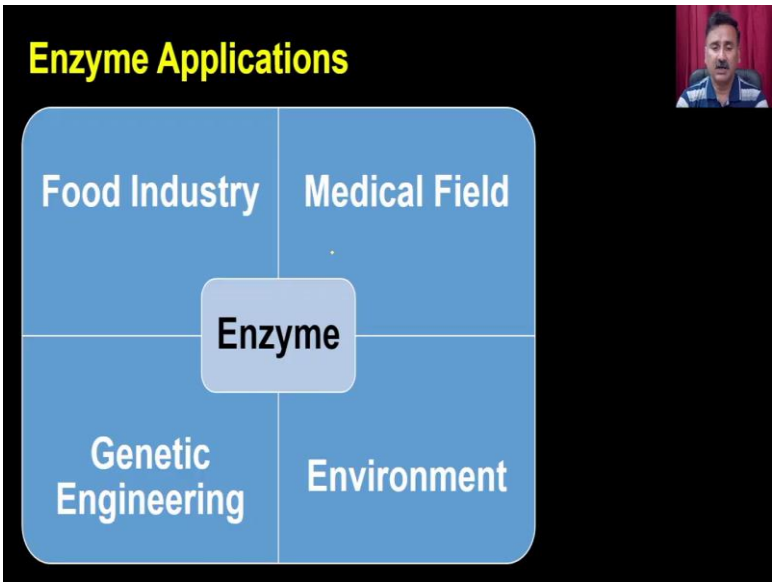

In the current lecture, we are going to discuss about the role of enzyme into the environment and how the enzymes are playing crucial role into the environment.

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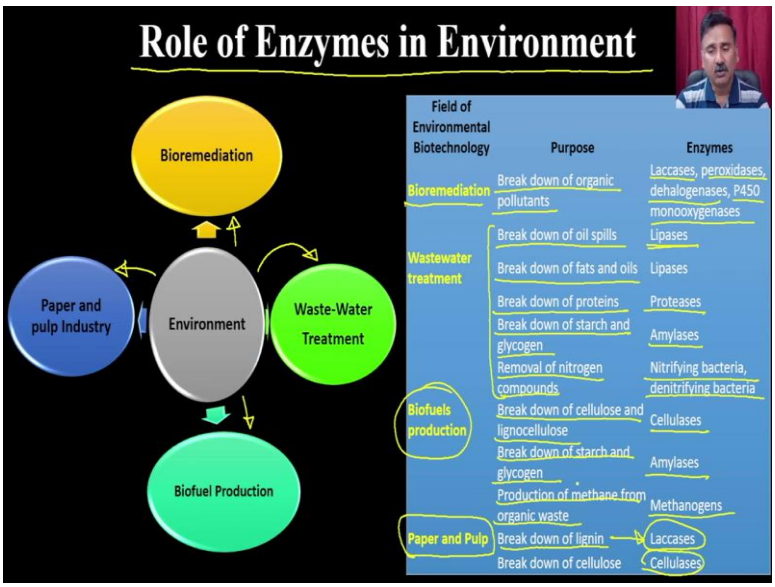

So, when we talk about the role of enzyme into the environment. The enzyme is actually going to have the role in the different aspects related to the environment. For example, you can have the role of enzyme into the waste water treatment, you can have the role in the enzyme in the bioremediations, you can have the role in into the paper and the pulp industry and you can also have the role into the biofuel productions.

So, when we talk about the bioremediations, you can have the breakdowns of the organic pollutants right, breakdown of the oil spillages and so on. And that has been always been done by the in different types of enzymes such as the laccases, peroxidases, and dehalogenases, P450, monooxygenases and so on.

And then we have the breakdown of the oil spillages that is being done by the lipases. And these are the different things what you can do in the waste water treatments. So, when we talk about the waste water treatment, you can have the breakdown of the oil spillages, breakdown of the fat and oils, breakdowns of the proteins, breakdowns of starch and glycogens, removal of nitrogenous compounds.

And that all been catalyzed by the different types of enzymes such as lipases, proteases, amylases and nitrifying bacteria or denitrifying bacterias. Then we have the role of enzyme into the biofuel productions. So, you can have the breakdowns of the celluloses and lignocelluloses, breakdowns of the starch and glycogens, then production of methane from the organic waste. This is being done by the different enzymes such as cellulases, amylases and methanogens. Then we also have the role of enzyme into the paper and the pulp industry.

So, where you can have the breakdown of the lignin or the breakdown of cellulose. So, breakdown of lignin is being catalyzed by the enzyme which is called as laccase and the breakdown of the cellulose is the crystallized by the enzyme of cellulases. So, we are not going to discuss all these enzymes, but we will take up few of the classical enzymes and how you can be able to produce these enzymes, how you can be able to utilize these enzymes for the for this particular purpose.

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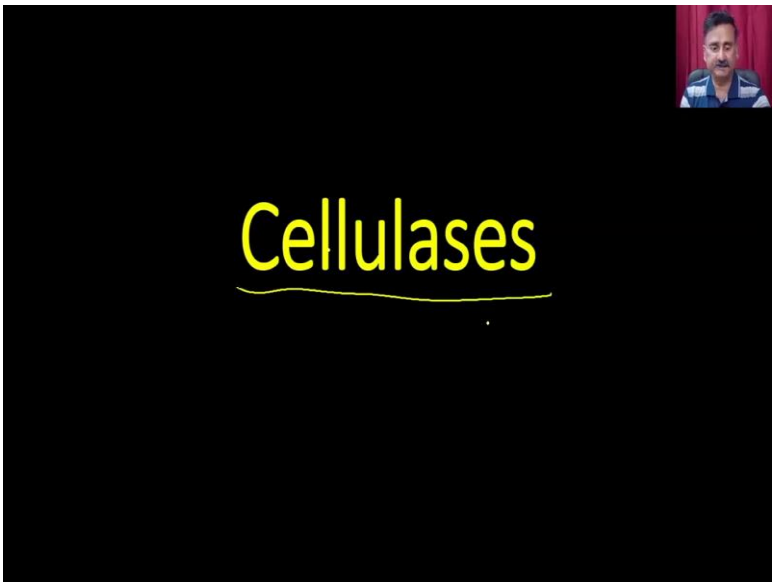

So, the first enzyme is the cellulases.

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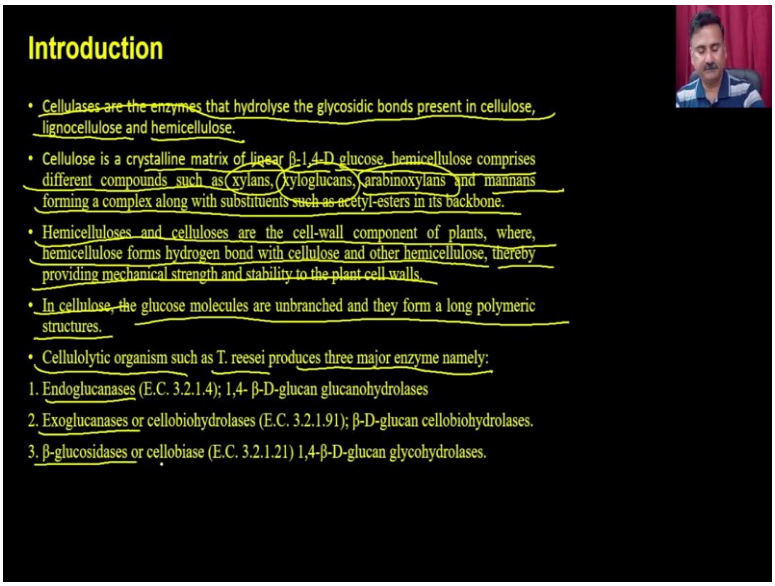

So, cellulases are the enzyme that hydrolyse the glycosidic bond present into the cellulose, lignincellulose and the hemicellulose. Cellulose is a crystalline matrix of linear betas 1, 4 glucose, hemicellulose compromise this different compounds such as xylans, xyloglucans, arabinoxylans and the mannans.

Forming a complex along with the substituents such as acetyl ester in its backbone. Hemicellulose and cellulose are the cell wall component of the plant where

hemicellulose forms the hydrogen bond with the cellulose and other hemicelluloses, thereby providing the mechanical strength and stability to the plant cells.

In cellulose, the glucose molecules are unbranched and they form a linear polymer structures. Cellulolysic cellulolytic organism such as T. reesei produces three major enzymes namely the endoglucanases, exoglucanases and the beta glucosidases or cellobiases.

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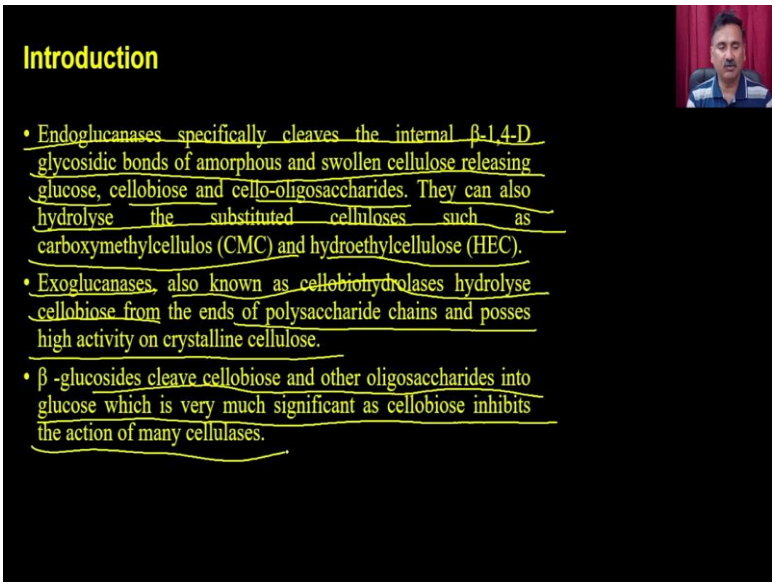

Endoglucanases specifically cleaves the internal beta-1-4-D glycosidic bond of amorphous and swollen glucose releasing a glucose molecules, cellobiose and cello-oligosaccharides. They can also hydrolyze the substituted cellulose such as carboxymethylcellulose and the hydroxyethylcellulose.

Exoglucanases also known as the cellobiohydrolases, hydrolyses the cellobiose from the end of the polysaccharide chain and possesses the high activity on the crystalline celluloses. Beta-glucosides cleaves the cellobiose and other oligosaccharides into the glucose which is very much significant as cellobiose inhibits the action of many cellulases.

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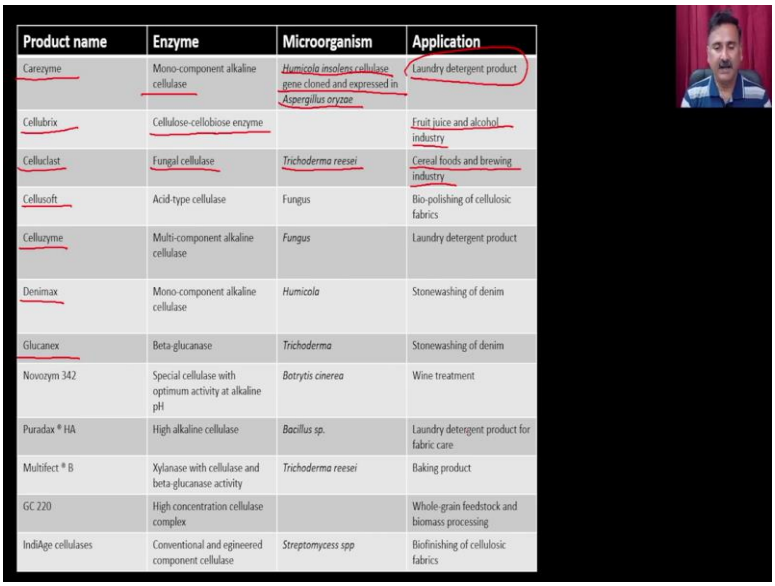

Product name	Enzyme	Microorganism	Application
Carezyme	Mono-component alkaline cellulase	*Humicola insolens* cellulase gene cloned and expressed in *Aspergillus oryzae*	Laundry detergent product
Cellubrix	Cellulose-cellobiose enzyme		Fruit juice and alcohol industry
Celluclast	Fungal cellulase	*Trichoderma reesei*	Cereal foods and brewing industry
Cellusoft	Acid-type cellulase	Fungus	Bio-polishing of cellulosic fabrics
Celluzyme	Multi-component alkaline cellulase	*Fungus*	Laundry detergent product
Denimax	Mono-component alkaline cellulase	*Humicola*	Stonewashing of denim
Glucanex	Beta-glucanase	*Trichoderma*	Stonewashing of denim
Novozym 342	Special cellulase with optimum activity at alkaline pH	*Botrytis cinerea*	Wine treatment
Puradax ® HA	High alkaline cellulase	*Bacillus sp.*	Laundry detergent product for fabric care
Multifect ® B	Xylanase with cellulase and beta-glucanase activity	*Trichoderma reesei*	Baking product
GC 220	High concentration cellulase complex		Whole-grain feedstock and biomass processing
IndiAge cellulases	Conventional and egineered component cellulase	*Streptomycess spp*	Biofinishing of cellulosic fabrics

These are the different types of products what are being developed for. So, you can have the carezyme. So, these are the monocomponent alkaline cellulases they are actually going to be produced by the humicola insolens cellulase gene cloned and expressed in the aspergillus oryzae. And they are being used for the laundry detergent products, ok.

Then you have the cellubrix. Cellubricks is a cellulose cellobiose enzyme and it is actually going to be used in the fruit juice and alcohol industry, then we have the celluclast which has a fungal cellulose and it is being produced by the trichoderma ressei and it is required for cereal food and brewing industries.

Then you have cellusoft, celluzymes all these enzymes and they all have the significant role in the different types of industries and we can have the you know. So, you can actually be able to read go through with these kind you know the role of these enzymes and other kinds of things and.

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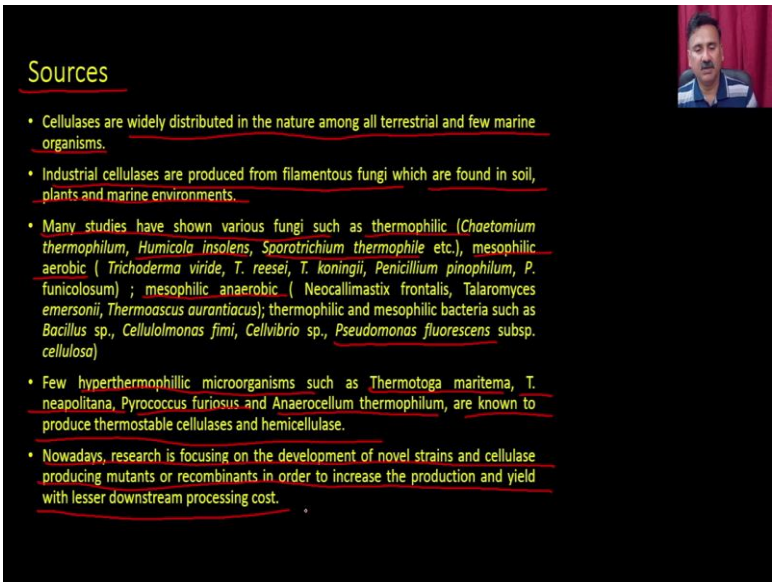

So, as per the sources is concerned if you want to develop the cellulases, cellulases are widely distributed in the nature among all terrestrial and few marine organisms. Industrial cellulases are produced from the filamentous fungus which are found into the soil, plants and marine environments.

Many studies have shown various fungi such as thermophilic, humicola insoles, sporotrichium, mesophilic aerobic and [FL] mesophilic anaerobic and as well as so pseudomonas fluorescens and all that are actually going to be having a significant role in the production of the celluloses.

Few hyperthermophilic organism such as thermotoga, maritema, or thermotoga neapolitana, pyrococcus and the anaerocellum thermophilum are known to produce the thermostable cellulases and hemicellulases. Nowadays, research is focusing on the development of novel strains and cellulase producing mutants or recombinant in order to increase the production and the yield with lesser downstream processing cost.

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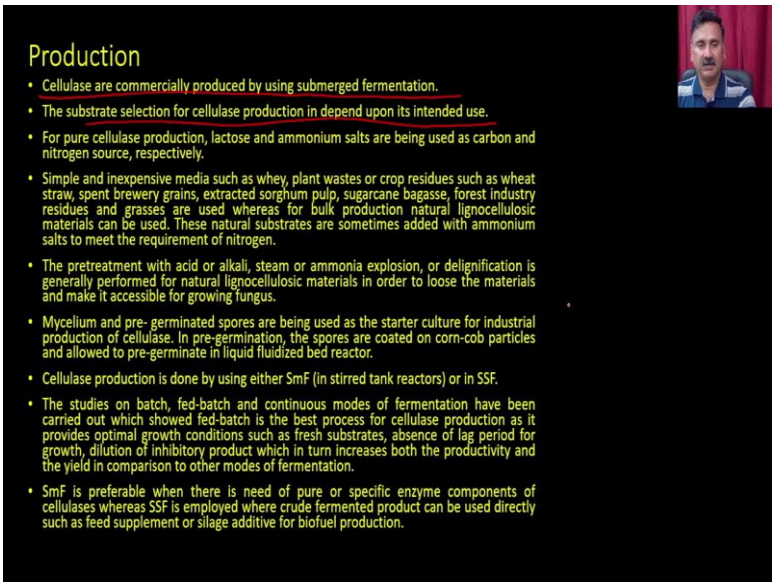

How you are going to produce? So, cellulase are commercially produced by the many submerged fermentation techniques. The substrate selection for the cellulase production depend upon it is about its intended use. The pure cellulase production lactose and ammonium salts are being used as the carbon and the nitrogen source respectively.

So, these are the details how you can be able to produce T cellulose into the submerged fermentations. Once you produce the cellulose into the organisms, you are actually going to isolate the cellulases and you are going to characterize the cellulases.

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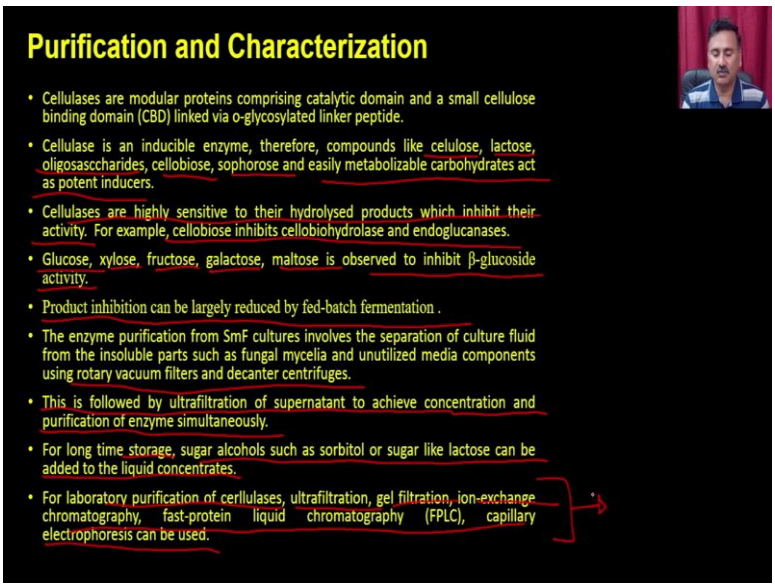

So, cellulases are modular proteins comprising the catalytic domain on a small cellulose binding domain linked via o-glycosidic linker peptides. Cellulases is a inducible enzyme therefore, compound like cellulose, lactose, oligosaccharides, cellobiose, sophorose and easily metabolizable carbohydrate act as potent inducer.

Cellulases are highly sensitive to their hydrolyzed products which inhibits their activity. For example, cellobiose inhibits cellohydrolase and celloglucanase. Glucose, xylose, fructose, galactose, maltose is observed to inhibit the beta glucoside activity. Product inhibition can be largely reduced by the fed back fed batch fermentations.

The enzyme purification from the SmF's cultured involves the separation of culture fluid from the insoluble parts such as fungal mycelia and media component using the rotatory vacuum filter and decanter centrifugations. This is followed by the ultrafiltration of supernatant to achieve the concentration of the purification of the enzyme.

For long time storage, the sugar alcohol such as orbital or sugar like lactose can be added to the liquid concentrates. For laboratory purification of cellulases, ultrafiltration, gel filtrations, ion exchange chromatography, FPLC, capillary electrophoresis can be used. All these we have discussed in detail. So, you can actually be able to go through the content and you will understand that how you can be able to use the cellulases, how you can be able to use the different chromatography techniques to purify the cellulases.

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Now, let us move on to the next enzyme and the next enzyme is the chitinases. So, chitinases are the enzyme which are actually going to degrade the you know the component which is called as chitin.

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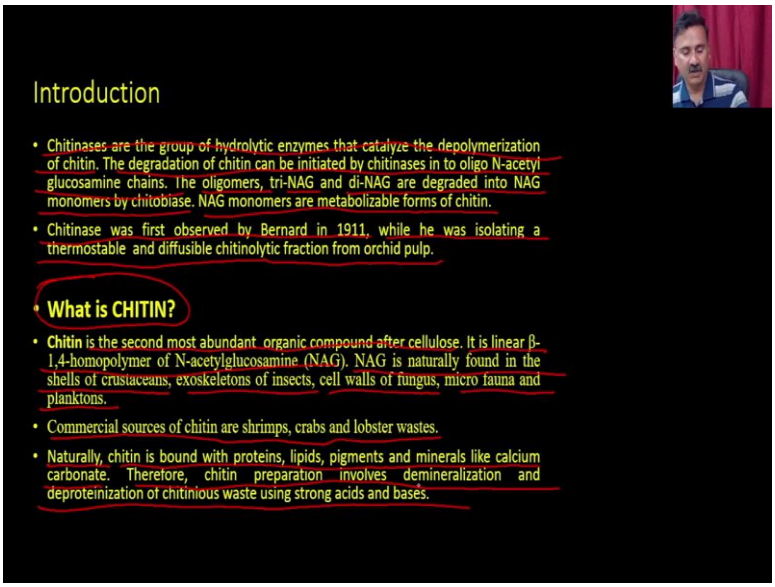

So, chitinases are the group of hydrolytic enzyme that catalyzes the depolymerization of the chitin. The degradation of chitin can be initiated by chitinases into the oligo N-acetyl glucosamine chain. The oligomers trinag and dinag are degraded into nag monomers by chitobiase and nag monomers are metabolized form of chitin.

Chitinases are first observed by Bernard in 1911 while he was isolating a thermostable and diffusible chitinolytic fraction from the orchid pulps. So, question is what is the chitin, ok. So, chitin is the second most abundant organic compound after cellulose it is a linear beta 1, 4-homopolymer of NAG.

NAG is a naturally found into the shells of the crustaceans, exoskeleton of the insects, cell wall of the fungus, micro fauna and planktons. Commercial sources of chitins are shrimps, crabs and lobster. Naturally chitin is found in the proteins, lipids, pigments and the mineral like calcium carbonate. Therefore, chitin preparation involve the demineralization and the deproteinization of chitinious waste using the strong acid and bases.

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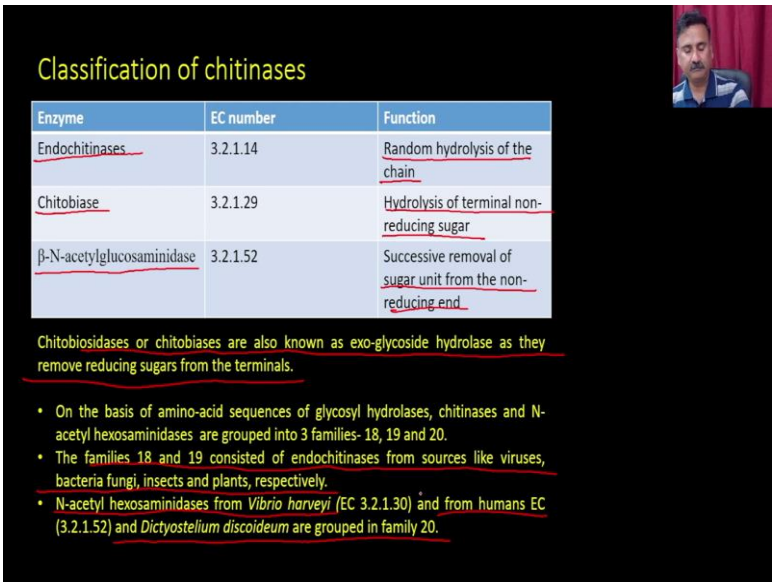

Now, there are three different types of chitinases. So, you can have the endochitinases, you can have the chitobiase and then you can have the beta-N-acetylglucosaminidase and the endochitinases are the random hydrolysis of the chain. Then we have the chitobiase which hydrolysis, the terminal, non reducing sugars and the beta-N-glucosaminidase is a successful removal of sugar units from the non-reducing ends.

Chitobiase or the (Refer Time: 14:06) are known as the exoglycosidase hydrolysis. They remove the reducing sugar from the terminals. On the basis of amino acid sequence of the glycosyl hydrolases, chitinases and N-acetylglucosamines are grouped into three family. Three families 18, 19 and 20.

The family 18 and 19 consist of the endochitinases from sources like virus, bacteria, fungi, insects and plants N-acetyl hexosaminidases from the vibro harveyi and from the humans and the dictyostelium discoideum are grouped into the family of 20.

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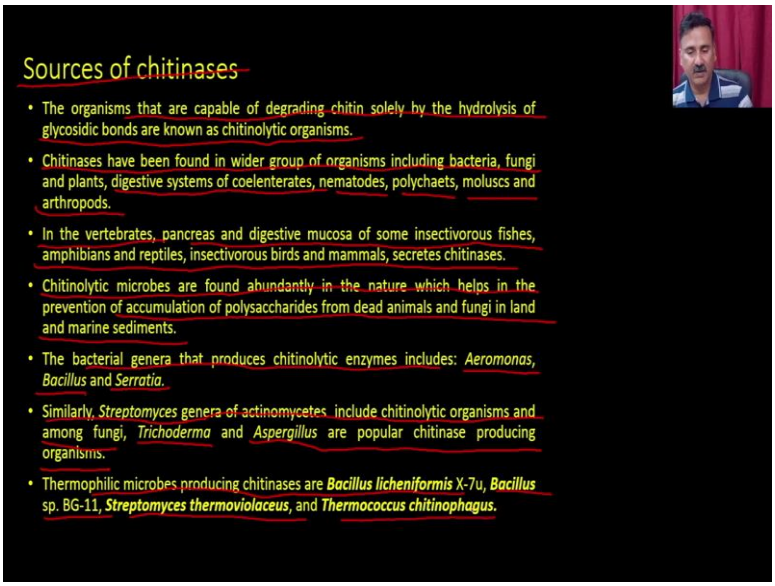

Sources of the chitinases, the organism that are capable of degrading chitin solely by the hydrolysis of glycosidic bonds are known as the chitinolytic organisms. Chitinases have been found in the wider group of organisms including the bacteria, fungi, plants, digestive system of coelenterates, nematodes, polychaets, moluscs and arthdropods.

In the vertebrates, pancreas and digestive mucosa of some insectivorous fishes, amphibians and reptiles, insectivorous birds and animals secretes the chitinases. Chitinolytic microbes are found abundantly into the nature which helps in the prevention of accumulation of polysaccharides from dead animals and fungi into the land and marine sediments.

The bacterial genera that produce chitinolytic enzyme includes the aeromonas, bacillus and serratia. Similarly, the streptomyces, the genera of actinomycetes includes the chitinolytic organisms and among fungi. Trichoderma and aspergillus are popular chitinases producing organisms. Thermophilic micro-organism producing chitinases are bacillus licheniformis, bacillus BG-11 and the streptomyces thermoviolaceus and thermococcus chitinophagus.

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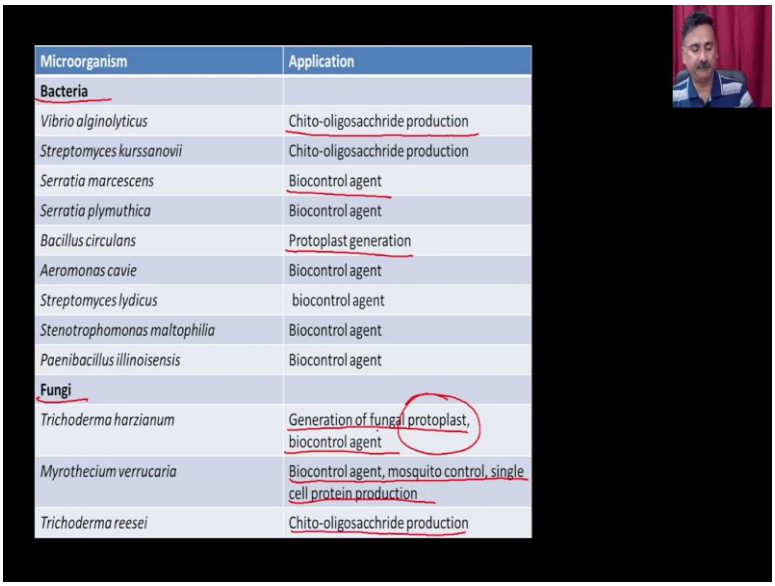

Then with these are the different types of microorganism which are producing the chitinases. So, you can have the bacterial species, you can have the fungal species and all these are actually producing the chitinase which are having the applications either into the chitin oligosacchride productions or the biocontrol agents or the protoplast generations or the generation of the fungal protoplast or biocontrol.

You know the chitinase sometime present in the as a component of the cell wall. So, if you use these, you know the chitinase from these organisms, they are actually going to degrade the cell wall and that is how they are actually going to produce the protoplast. Then we also been used to biocontrol agents, mosquito control, single cell production, protein productions and the chitin oligosacchride productions. How you are going to produce the chitinases?

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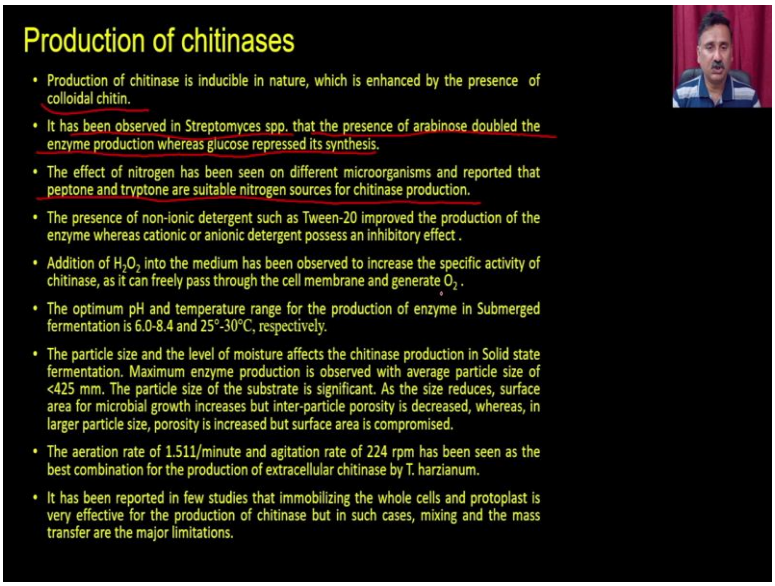

So, the production of chitinase is inducible in nature which is enhanced by the presence of the colloidal chitin. It has been observed in streptomyces species that the presence of arabinose double the enzyme production whereas, glucose represents its synthesis. The effect of nitrogen has been seen on different micro organism and reported that the peptone and the tryptone are the suitable nitrogen sources for the chitinase productions.

And these are the some of the conditions I have given so you can actually be able to go through the content and it will actually going to tell you that what are the conditions are you know, facilitating the production of chitinases.

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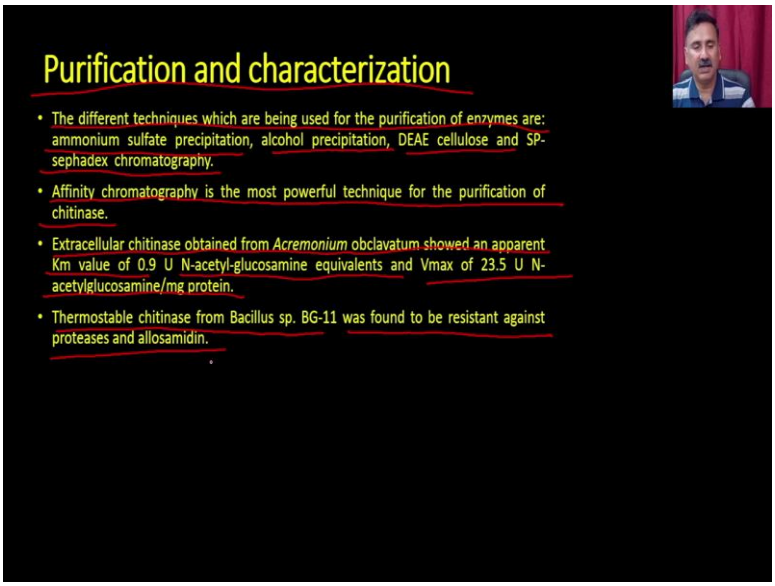

Then we have the purification and the characterization. So, the different techniques which are being used for the purification of enzymes are the ammonium sulfate precipitations, alcohol precipitations, DEAE cellulose and the SP sephadex chromatography. Affinity chromatography is the most powerful technique for the purification of the chitinases.

The extracellular chitinases obtained from the showed an apparent Km value of 0.9 units against the N-acetylglucosamine equivalents and Vmax of twenty point 23.5 units N-acetylglucosamine per milligrams of proteins. Thermostable chitinases from bacillus species BG-11 was found to be resistance against proteases and the allosamidin.

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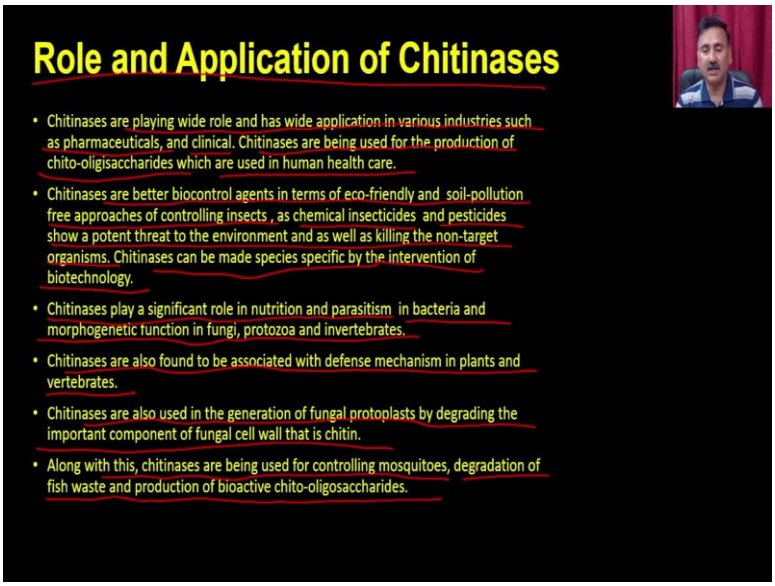

So, what is the role and the application of the chitinases? So, chitinases are playing widal role wide role and are wide application in various industries such as pharmaceuticals and the clinicals. Chitinases are being used for the production of the chito-oligosaccharides which are used in the human health care.

Chitinases are better biocontrol agent in terms of eco-friendly and soil-pollution free approaches of controlling the insects, as chemical insecticide and pesticides show a potent threat to the environment and as well as the killing the non-target organisms. Chitinases can be found species specific by the intervention of the biotechnology.

Chitinases play a significant role in nutrition in the parasitism in bacteria and morphogenetic function in the fungi, protozoa invertebrates. Chitinases are also found to be associated with defense mechanisms in the plants and vertebrates. Chitinases are also found in the generation of fungal protoplast by degrading the important component of the single fungal cell wall that is the chitin.

Along with this chitins are being used for controlling the mosquitoes, degradation of the fish waste and the production of bioactive chitin oligosaccharides. So, chitin is a very very important enzyme which actually can be used to control the level of the chitin into the environment.

And that is how you can actually be able to control the many processes such as for example, you can actually be able to control the mosquito production or level of mosquito. Because if you treat the things with the chitinase it is actually going to degrade the cell wall or the exoskeleton of the these microorganisms.

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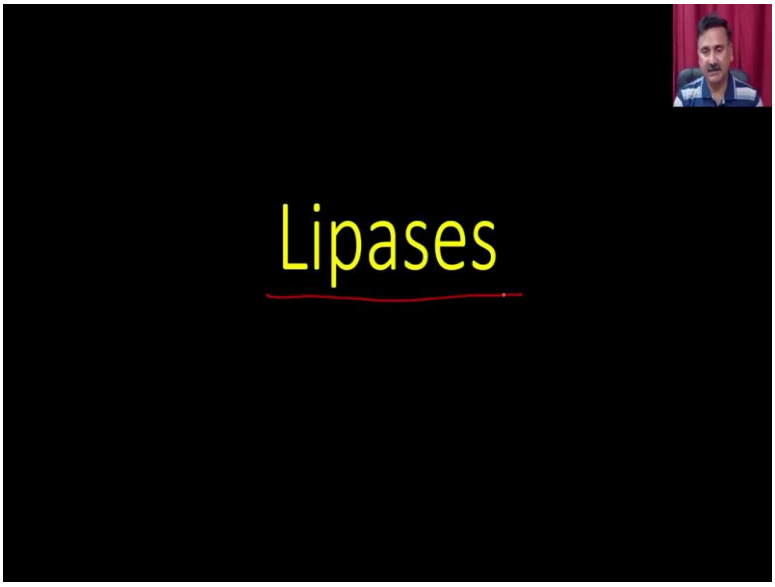

Now, let us move on to the next enzyme and the next enzyme is lipases.

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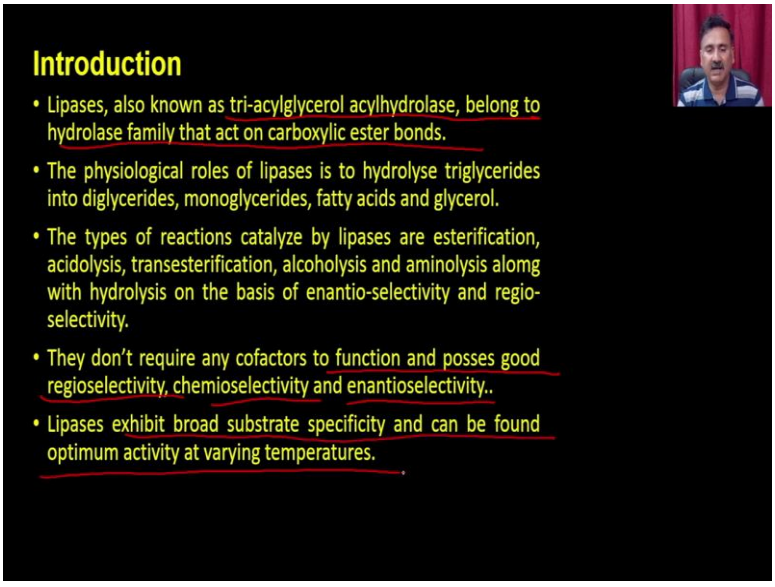

So, lipases are also known as the triacylglycerol acylhydrolase belonging to the hydrolase family or act on a carboxylic acid ester bonds. The physiological role of lipase

is to hydrolyze the triglycerides into the diglycerides, monoglycerides, fatty acid and glycerol. The type of reaction catalyze by lipases are esterification, acidolysis, transesterification, alcoholysis, and aminolysis along with hydrolysis on the basis of enantio-selectivity and regio-selectivity.

They do not; they do not require any cofactor to function and posses the good regioselectivity, chemioselectivity and enantioselectivity. Regioselectivity means they are actually can be able to select even the within the lipid molecule which lipid they have to select.

Chemioselectivity means they are actually be able to select the lipids molecule based on the chemical groups. And the enantioselectivity means they can actually be able to even select the isomeric forms of the lipids. Lipid exhibits the broad substrate specificity and can be found optimal activity at a varying temperatures.

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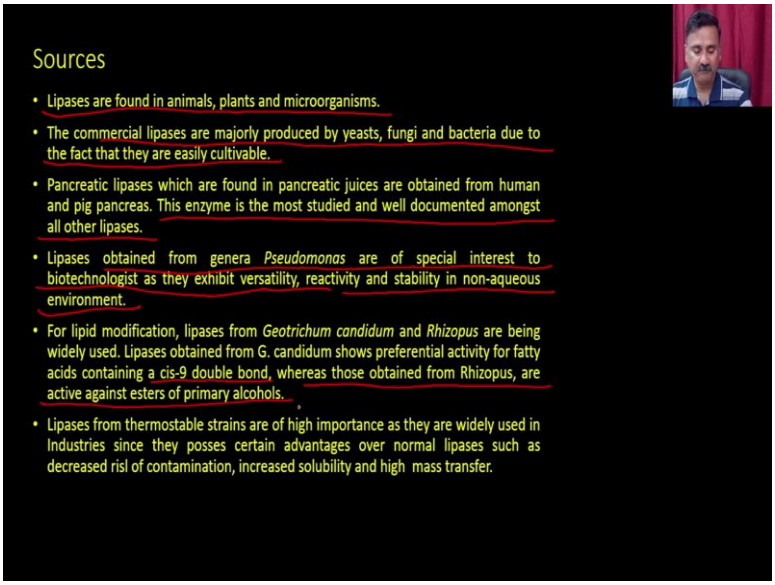

Sources: so lipases are found in animal plants and microorganism. The commercial lipases are majorly produced by the yeast, fungi and bacteria due to the fact that they are rarely cultivable. Pancreatic lipase which are found in the pancreatic juices are obtained from the human and pig pancreas. This enzyme is the most studied and well documented among all other lipases.

Lipases obtained from the pseudomonas are of special interest to the biotechnologist as they exhibit versatility, reactivity and stability in a non-aqueous environment. For lipid modifications, lipases from the rhizopus are being widely used. Lipases obtained from the shows preferential activity for fatty acid containing a cis-9 double bond, whereas those obtained from the rhizopus are active against the ester of the primary alcohol.

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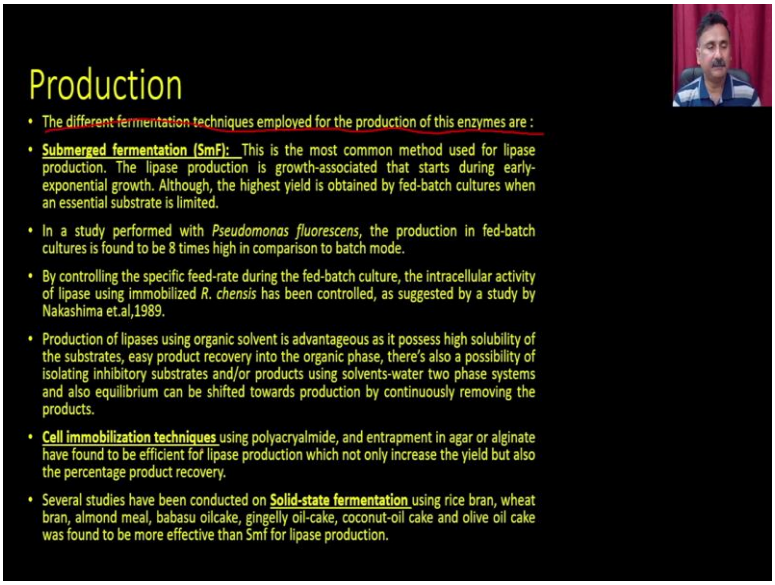

Production, so the different fermentation technique employed for the production of the enzymes are the submerged fermentation techniques or the cellular immobilization techniques.

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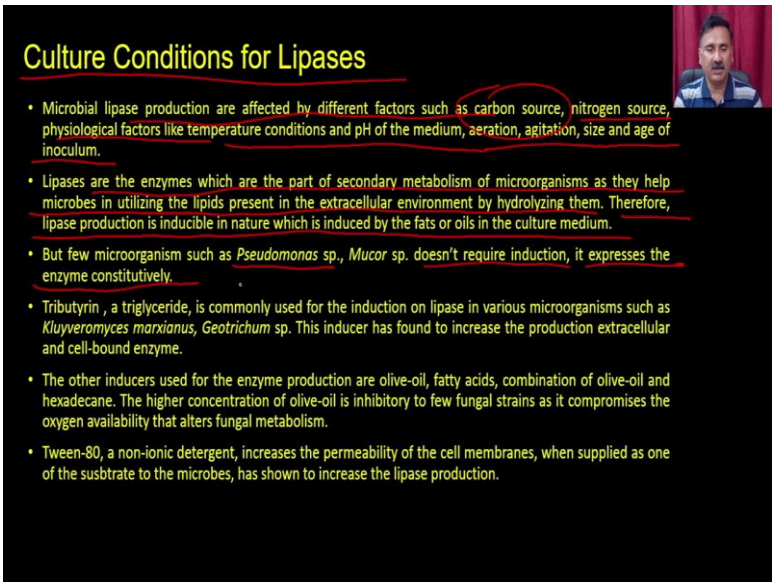

And there are specific culture condition for the lipases. So, you can have the microbial culture, (Refer Time: 23:02) lipase production affected by the different factors such as carbon source, nitrogen source, physiological factors such as temperature, pH, aerations, agitation, size and age of the inoculum.

Lipases are the enzyme which are part of the secondary metabolism of organism as they help in microbes in utilizing the lipid present in the extracellular environment by hydrolyzing them. Therefore, lipase production is inducible in nature which is induced by the fat or oil into the culture condition. But few microorganism such as pseudomonas does not require induction, it expresses the enzyme constitutively.

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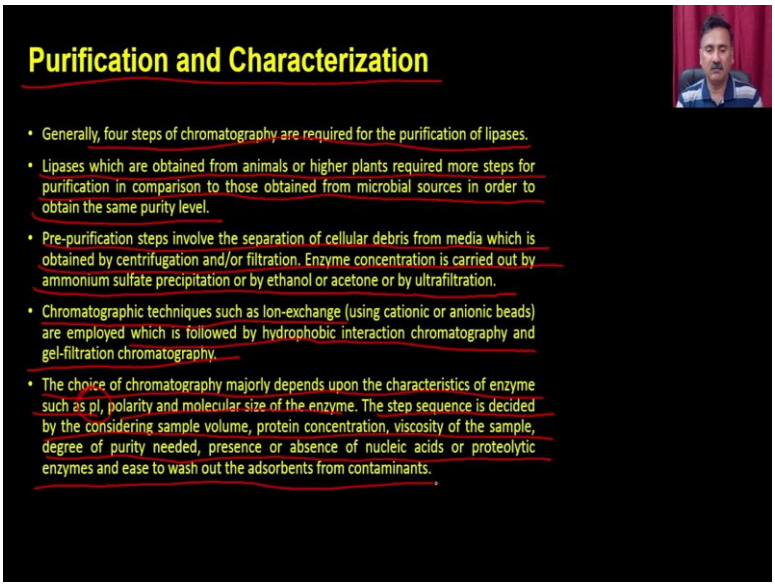

Then we have the purification and the characterization. So, generally four steps of chromatography are required for the purification of lipases. Lipases which are obtained from the animal or higher plant require more steps for purification in comparison to those obtained from the microbial sources in order to obtain the same purity level. Pre purification steps involved in the separation of the cellular debris from media which is obtained by centrifugation or filtration.

Enzyme concentration is carried out by the ammonium sulfate precipitation or by ethanol or acetone by ultrafiltration. Chromatographic techniques such as ion exchange which is followed by the hydrophobic interaction chromatography and the gel filtration chromatography. The choice of chromatography majorly depend on the characteristics of the enzyme such as (Refer Time: 24:34) point, polarity, molecular size of the enzyme.

The step sequence is decided by the considering sample volume, protein concentration, viscosity of the sample, degree of protein needed, presence and absence of nucleic acid or the proteolytic enzyme and ease to wash out the absorbent from the contaminants.

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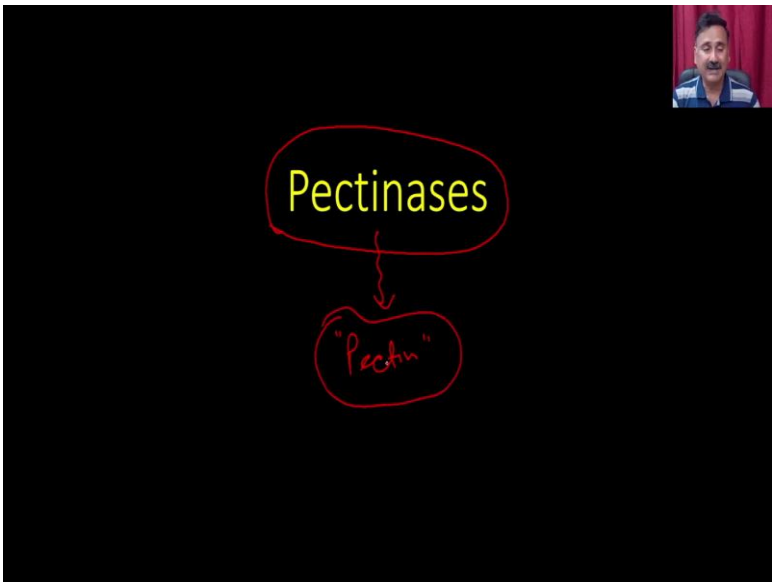

Then we also can talk about the another enzyme which is called as the pectinases. Pectinases are the enzyme which are actually going to degrade the very important cellular component of the cell wall which is called as pectin, right.

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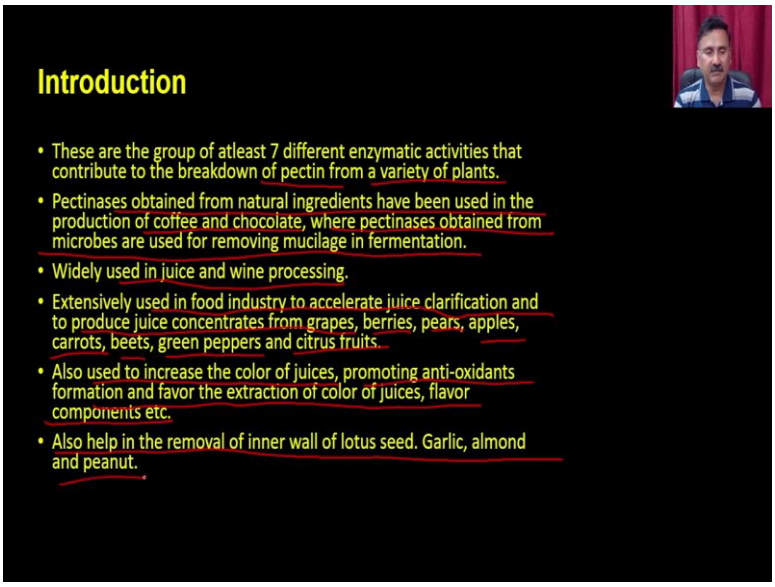

So, these are the group of at least 7 enzymatic activities that contribute to the breakdown of the pectin from the variety of plants. Pectinases obtained from the natural ingredients have been used in the production of coffee and the chocolates where pectinases obtained from the microbes are used for the removal of mucilages in the fermentations.

It is widely used in the juice and wine industry, extensively used in food industry to accelerate the juice clarification and to produce the juice concentrate from the grapes, berries, pears, apples, carrot, beets, green peppers and the citrus fruits. It also used to increase the color of juice promoting the antioxidant formation and favor the extraction of the color of juice favors components, flavor component etcetera, also helping the removal of inner wall of lotus seed, garlic, almonds and peanuts.

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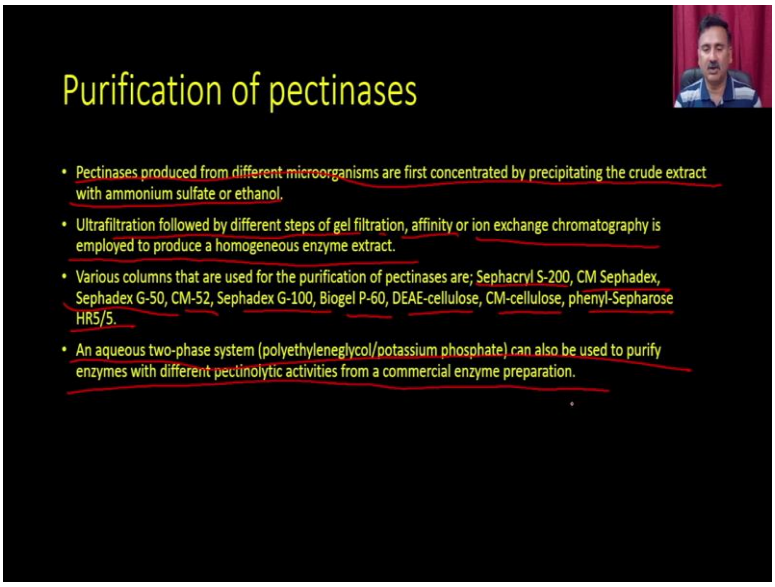

Sources the most common source of pectinases is the filamentous fungus aspergillus species which is produces the pectinolytic enzymes, de-esterifying and chain splitting enzymes. Also done from the tomato and oranges and there are other organisms also which are also known to produce the pectinases.

In the production you can actually have the submerged fermentations and you can also have the solid state fermentations. As far as the purification is concerned the pectinases produced from the different microorganisms are first concentrated by precipitating the crude extract with the amino sulfate. Then you have the ultrafiltration followed by the different steps of gel filtration, affinity chromatography, ion exchange chromatography and employed to produce the homogeneous enzyme extracts.

Various column that are used for the purification of pectinases are Sephacryl S-200, CM Sephadex, Sephadex G-50, CM-52, Sephadex G-100, Biogel P-60, DEAE cellulose, CM cellulose, phenyl-Sepharose HR 5 by 5. An aqueous two-phase system can also be used

to purify the enzyme with different pectinolytic activities from a commercial enzyme preparations.

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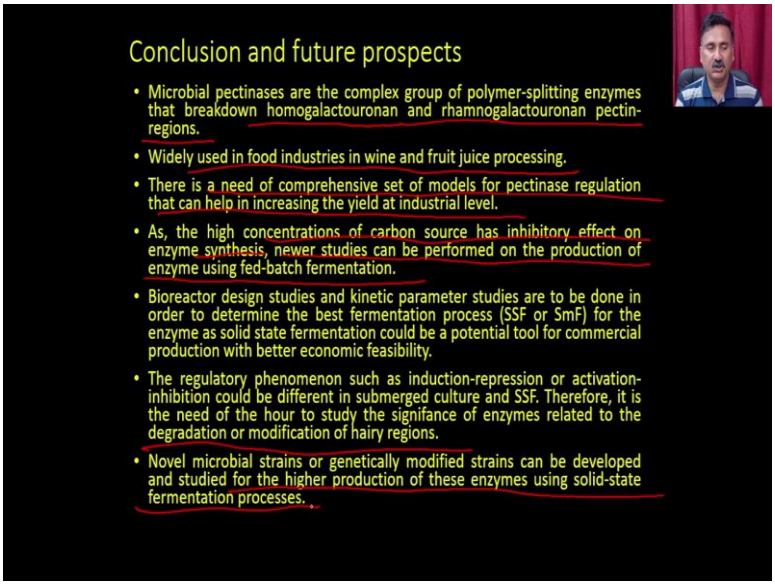

So, microbial pectinases are the complex group of polymers splitting enzyme that break down the homogalactouronan and pectin regions. Widely used in the food industry in the wine and fruit juice processing. There is a need of comprehensive set of model for pectinase regulation that can help in increasing the yield at the industrial level.

As the concentration of the carbon source has inhibitory effect of the enzyme synthesis, newer studies can be performed on the production of enzyme using the fed batch fermentations. The regulatory phenomena such as induction repression or the activation inhibition can be different in the submerged culture and the solid state fermentations.

Therefore, it is need of the hour to study the significance of enzyme related to the degradation of degradation or the modification of the hairy regions. Novel microbial strain or genetically modified strain can be developed and studied for the higher production of these enzyme using the solid state fermentation processes.

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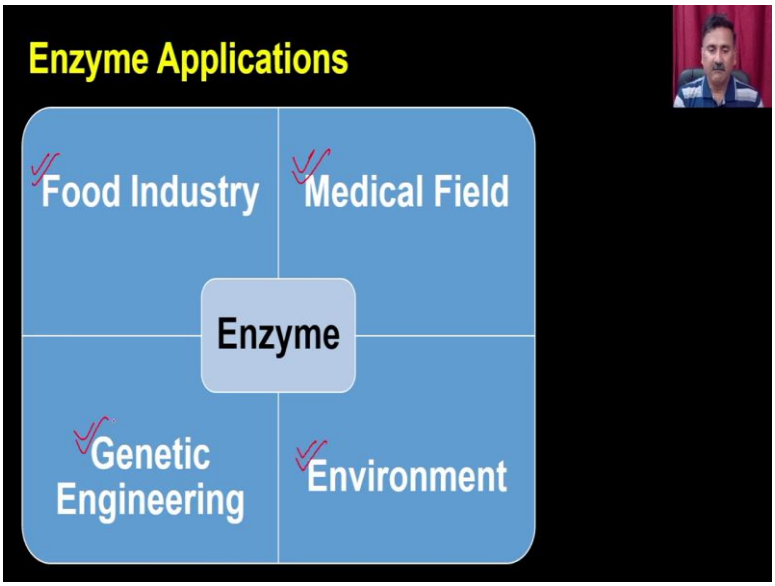

So, this is all about the enzyme applications into the food industry, into the medical field, into the genetic engineering and into the environment. If you recall genetic engineering, the role of enzyme into the genetic engineering, we have discussed in detail when we are talking about how you can be able to use a clone in the enzyme, how you can actually be able to do the polymer, you know PCR you know all other kinds of things.

So, that is why I have not taken that part and discussed in this particular module. So, with this, I would like to conclude my lecture here. In our and in this lecture, we have discussed about the role of enzyme into the food industry, medical field and the environment and as well as the genetic engineering.

And since this is the last lecture of this particular course, we have already been discussed in detail about the various aspects of the enzymes we have discussed. We started very basic with the with very basic information about what are the enzymes, how the enzymes are facilitating the conversion of the substrate into the product and so on. And then we have also discussed about the nomenclature and classifications and so on.

The mechanism of enzyme actions and then we also discussed how you can be able to solve the structure of the enzyme. So, we discussed about the experimental method and where we have discussed about the NMR and as well as the X-ray crystallography. And then we also discussed about the non-experimental methods. So, we discussed about the molecular modeling and how you can be able to use the modular 9 version 9 to model the

enzyme and how you can be able to test or validate whether the model design is of good quality or not.

Subsequent to that we have also discussed about how you can be able to study the enzyme catalyzed reactions, what are different types of reactions. So, we discussed about the carbohydrate metabolism, we discussed about the beta oxidations, amino acid metabolisms and so on. And subsequent to that, we have also discussed about how you can be able to study the enzyme substrate interactions.

So, we discussed about the spectroscopic approaches such as the difference spectroscopy, we discussed about the ITC, we discussed about the SPR and so on. And once we understand all these, we discussed about the how you can be able to study the kinetics of the enzyme substrate interactions, how you can be able to develop the enzyme assays and how you can be able to develop the enzyme inhibitors.

And once we have developed all these, we have discussed about the significance of the enzyme into the human welfare and how you can be able to use the enzyme for the different aspects of the applications. So, with this, I would like to conclude my lecture here and I hope that you might have like the content of this particular course and you will could get some, you know you it could have been beneficial for you for the long run. So, with this, I would like to conclude my lecture here.

Thank you.