**Course on Industrial Biotechnology**
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**Indian Institute of Technology Kharagpur**
**Lecture 47**
**Module 10**
**Alpha Amylase Production**

Welcome back to my course that industrial biotechnology now in the less in last lecture I I tried to cover spirulina one thing I I forget to mention that that you know that we we we have one book that is algal Biorefinery An Integrated Approach.

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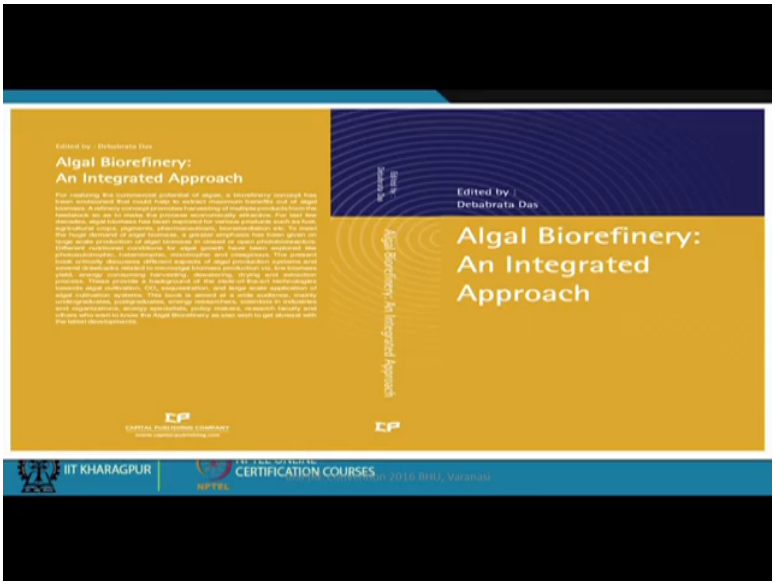


So I I suggest that if you want the information about the algal that production process we can get the (())(00:50) information in this book. Now let me come back to the this lecture that we are we know that different enzymes is very much used in the day to day life and one of the very important enzyme is alpha amylase which has lot of applications in the in the different industry and also it is used as a for medicinal not only used for in some food making industry but also used in the medicinal for in medicinal purpose.

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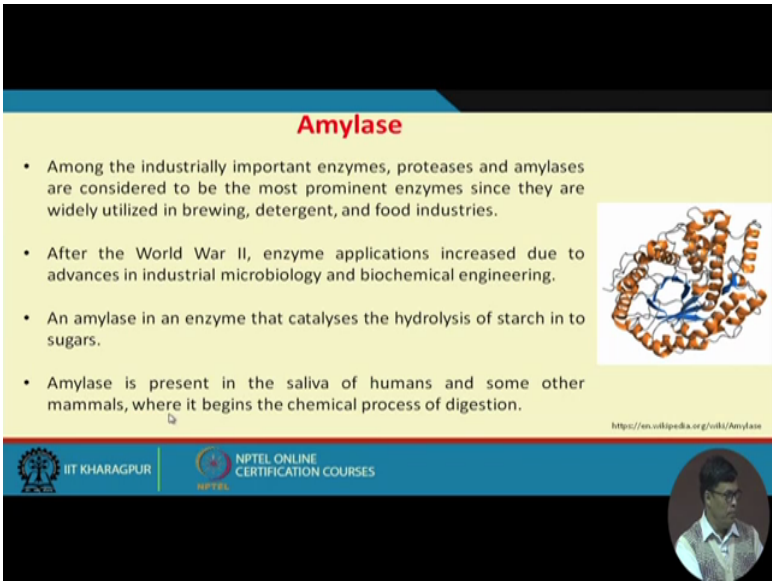


Now this alpha amylase we have this among the industrial important enzymes like protease that amylase are considered the most prominent enzymes since their widely utilized in brewing, detergent and food industry because we know that in detergent industry I told you that sometimes we have some blood clot in the in in our cloth and this is blood is kind of protein molecule.

So if we use some kind of protease enzyme that will clean this cloth and sometimes we have some kind of strain due to some fat type of material to use (())(02:13) that also do the it can remove the strain and sometimes the starchy material also has some kind of colour their contribution to our cloth that also remove by amylase.

So this is exactly what is mentioned here that used in the detergent, food industry is largely used I think we are going to talk about the high fructose corn syrup production that their I shall tell you more and brewing industry already we have seen how brewing industry because the first steps in the brewing industry is the malting process and through the malting process actually we we develop lot of hydrolytic enzymes like alpha amylase and protease this enzymes are produced during the uhhh germination process.

After the World War 2 the enzymes application increases due to the advances in industrial biotechnology and biochemical engineering and amylase is an enzyme that catalyse and hydrolyse the starch into sugar. And amylase is present in the saliva of human because we know our saliva contains the kind of amylase enzymes some other mammals where it begins the chemical chemical process of digestion.

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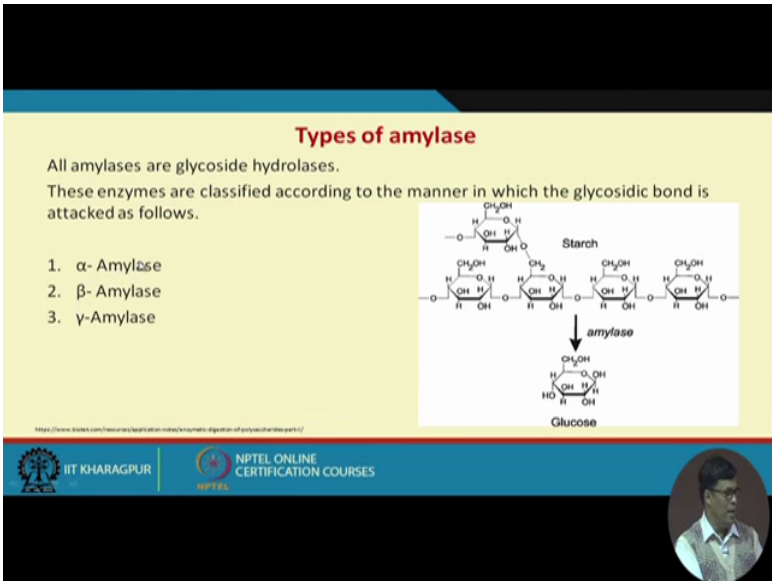


So we have three different amylase type of amylases we have alpha amylase, beta amylase and gamma amylase we will be concentrating on mostly on alpha amylase today.

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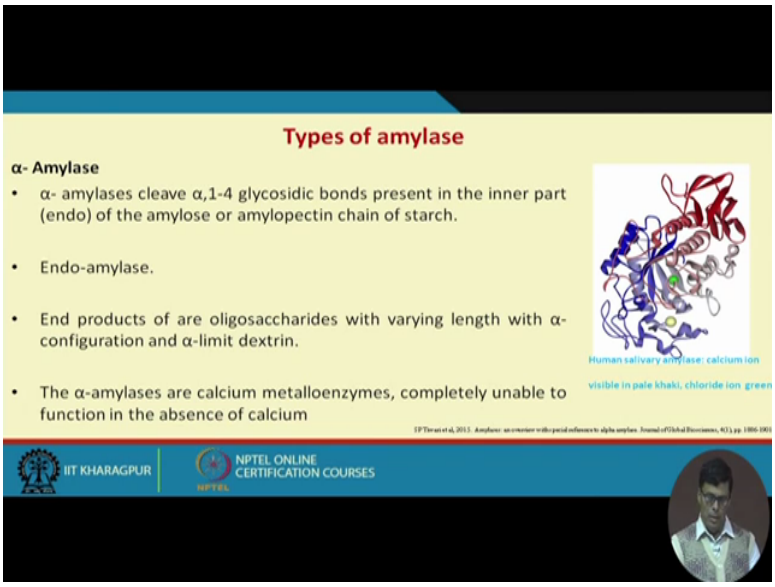


And if you look at alpha amylase alpha amylase is cleaved alpha 1-4 linkage we know the starchy molecule is is a polymer of glucose and they binds with each other by alpha 1-4 linkages and this is alpha amylase cleave the alpha 1-4 glycosidic bond present in the inner part of the amylose amylopectin chain of the starch. Starch has two type of chain one is called amylase.

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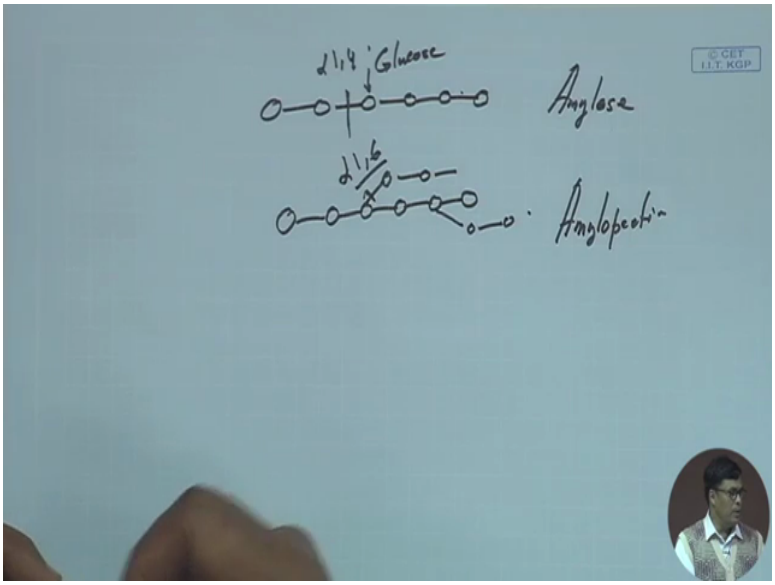


Amylase means the starch molecules they they will they will they will they will they form this is the chain this is the chain molecules and this is amylose and this is glucose molecule, this is the polymer of the glucose.

Now in case of amylopectin we have the branch branching we have here we have we can have some branching here, so we have this is called amylopectin. So this bond is alpha 1-4 linkage and this bond is alpha 1-6 linkage. So they have different type of linkage that we have and alpha amylase calcium is very much required for the activity of the enzymes we observe the thermostability of the alpha amylase enzyme increases to a great extent in presence of calcium ion.

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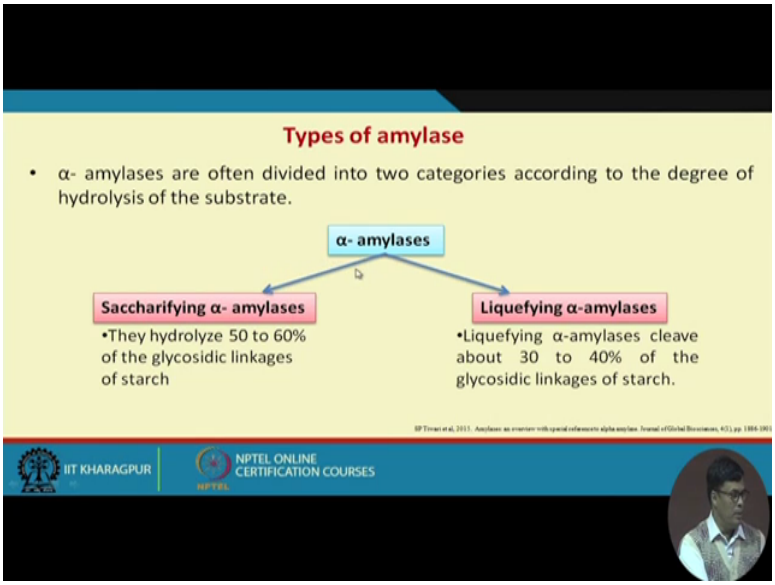

So alpha amylase can be used for two purpose saccharifying alpha amylase one is liquefying alpha amylase The saccharifying alpha amylase they hydrolyse 50 to 60 percent of glycosidic linkage of starch, liquefying alpha amylase cleave about 30 to 40 percent of glycosidic linkages of the starch.

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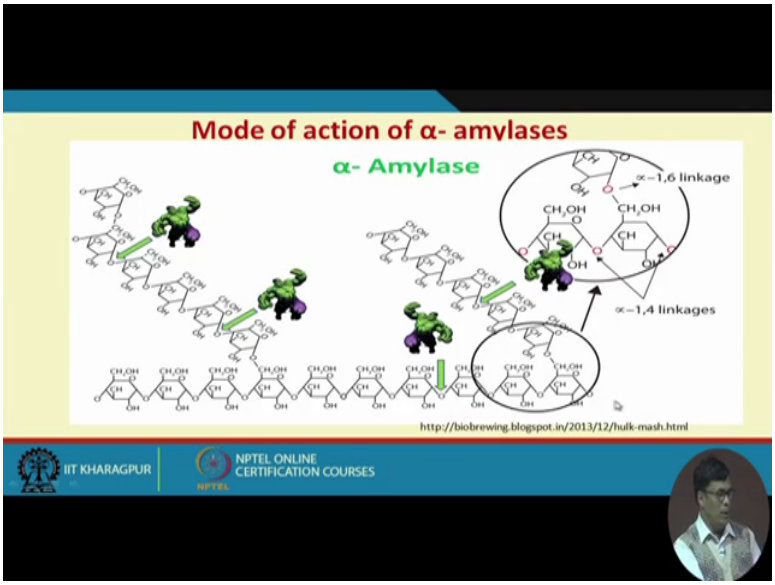


There is the two types of alpha amylases and this is they call endo endo endoamylase endoamylase means that this can randomly attack the starch molecules at any position, you see that any position here here here at the so in process we produce oligosaccharide oligosaccharide means it is dimer, trimer, tetramer different uhhh this hexose molecules they can combination they produce the different type of oligosaccharide that we get the as a product due to the action of this alpha amylase.

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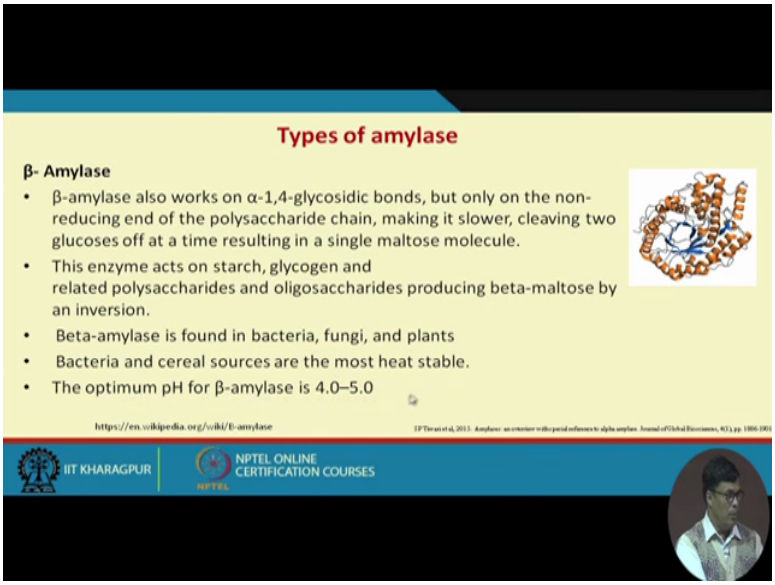


And then we have other types of amylase, the beta amylase the beta amylase are they walk alpha 1-4 glycosidic bond but only on the non-hydrogen non reducing end of the polysaccharide and making it slow and cleave two glucose off at a time resulting a single maltose molecule. So I can show you this it is beta amylase largely available in bacteria and fungi and pH is about 4 to 5.

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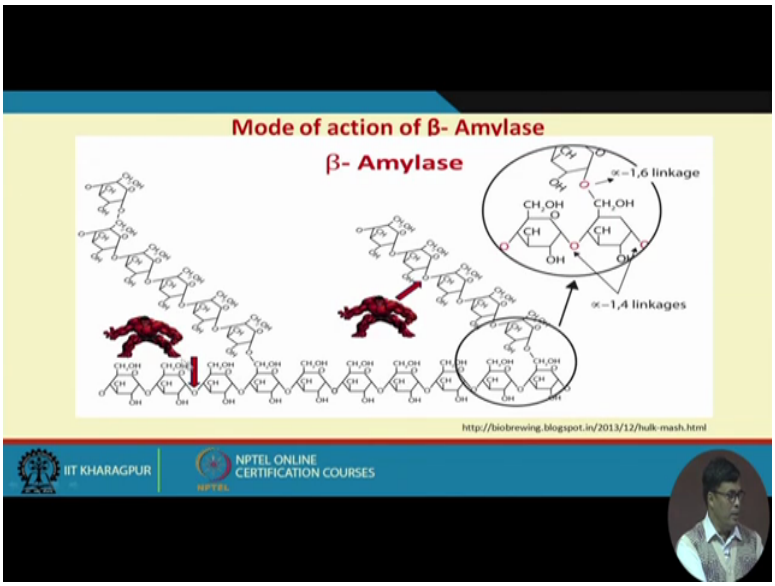


Now if you look at here that this is the non-reducing end and here the this this beta amylase so two glucose unit can comes out here also two glucose units, we know that this two glucose unit when they bind with alpha 1-4 linkage we call is the amylose.

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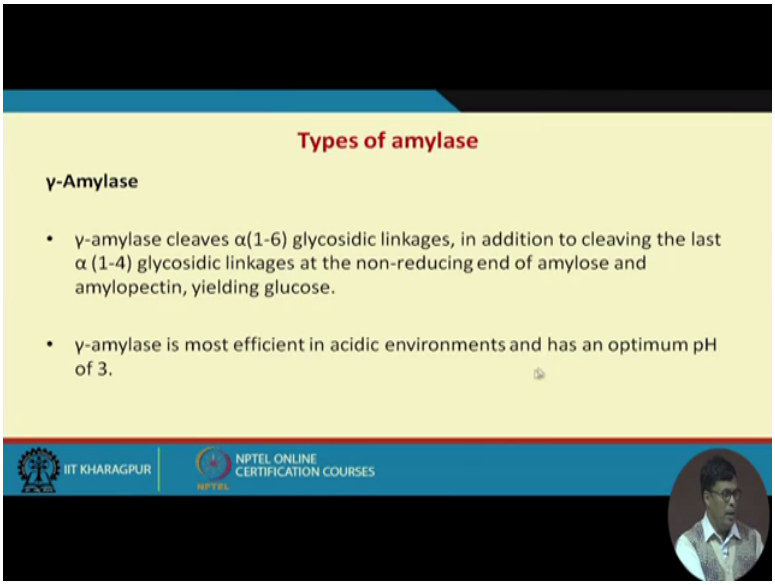


So this is amylose is the main product that then gamma amylase is a cleave the alpha 1-6 glycosidic I have already pointed out that this bond is alpha 1-6 glycosidic linkage and this is alpha 1-4 glycosidic linkage this is the difference that we have in an in addition to 1-4 linkage at the non-reducing end of amylose and amylopectin, yielding glucose. So gamma amylose is more efficient in acidic environment as an optimum pH of 3.

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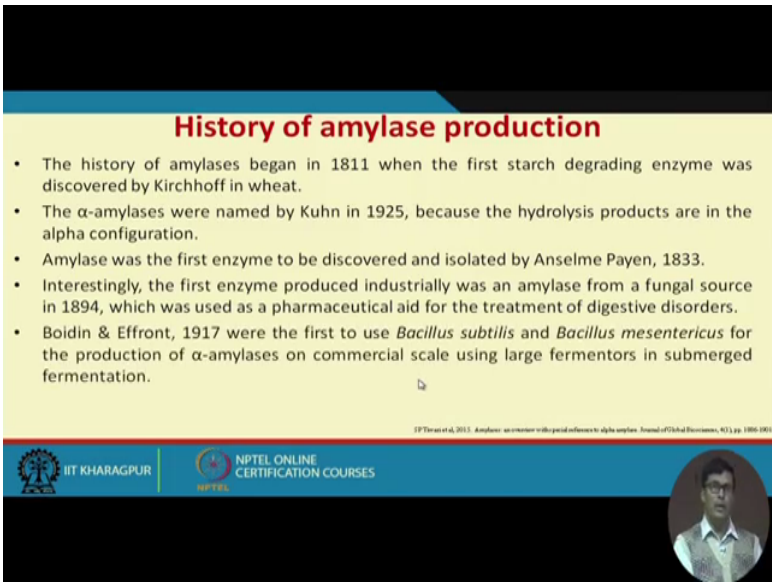


So history of amylose production is like this history amylose begin in the year 1811 when the first starch degrading enzyme was discovered by Kirchhoff in wheat, then alpha amylase was named at Kuhn in 1925 because the hydrolysis products are alpha having the alpha configuration, then amylase was first to enzyme to be discovered and isolated by Payen in

1833, then interestingly the first enzyme produced industrially was an amylase from fungal source in 1894, which was used pharmaceutical aid for the treatment of digestive disorders.

We know during I told you that the amylase has lot of medicinal applications when we use the we have digestive problem that doctor usually prescribe some kind of tonic which contains amylase and and protease that help in our digestion system, the and the Boidin and Effront in 1917 were the first to use the bacillus subtilis and bacillus mesentericus for the production of alpha amylase on commercial scale using the large fermentors by submerged fermentation process.

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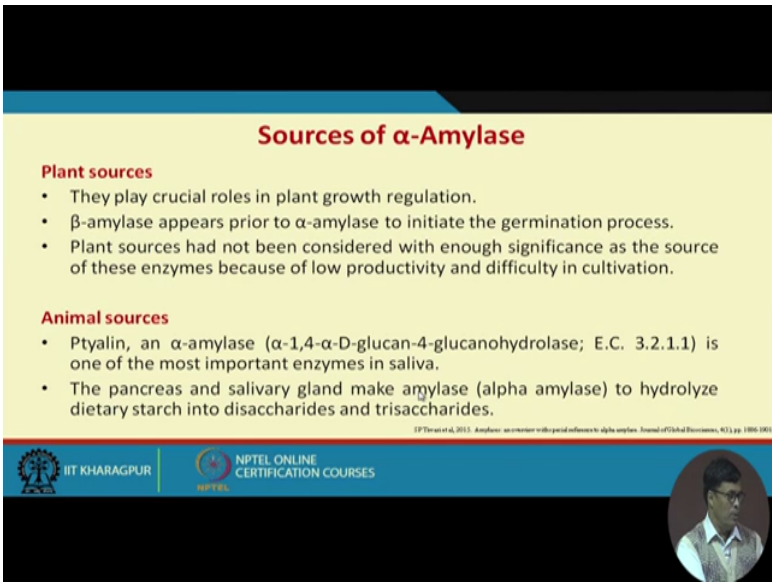


The industrial production of alpha amylase the plant source this is they played crucial role in the plant growth regulation, beta amylase appears prior to alpha amylase to initiate the germination process. Plant sources are are not being considered with enough significance as the source of this enzyme become low productivity and difficult to cultivate, the animal source we have we have like Ptyalin that is an alpha amylase that is one of the most important enzyme in the in the saliva that I told you in the saliva we have this amylase enzyme when we take the food then mix with our with our food and starchy material will degrade.

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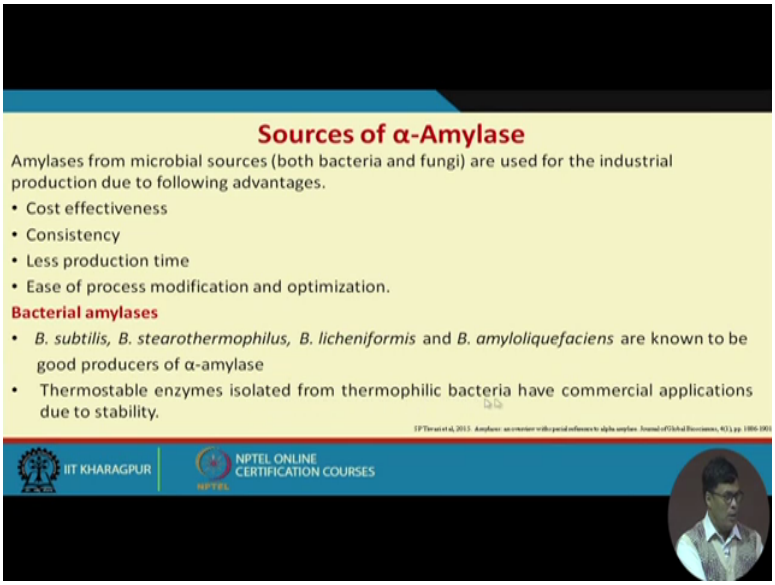


Then the alpha amylase based is produced from microbial source both from bacteria and fungi this for industrial production it is cost effective, consistency, less production time, ease of process modification and optimization. The bacterial amylases we have several we have bacillus subtilis, we have bacillus stearothermophilus, we have bacillus licheniformis, bacillus amyloliquefaciens are known to produce a good amount of alpha amylase. Thermostable enzymes isolate from thermophilic bacteria having the commercial application due to stability.

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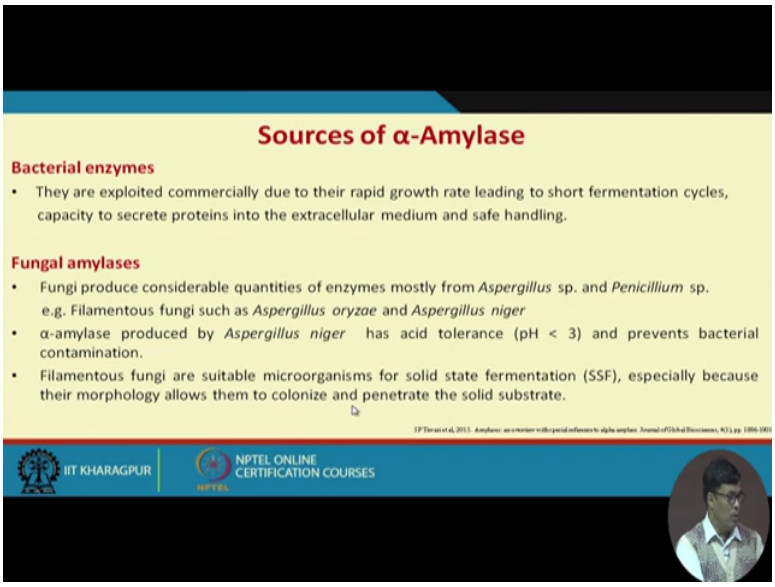


Now bacterial enzymes there are exploited commercially due to their rapid growth leading to shorter fermentation cycle capable of secreting the protein into the extra extra cellular media

and safe handling. Now here I want to point out that all the hydrolytic enzymes produced by the microorganisms they are usually the extra cellular in nature so you that does not presence inside the cells. So it presence in the fermentation media so you can fully you can separate the cell mass and in the fermentation broth you will get the in the filtered you will get that that enzymes, so you can purify it.

So so this is the enzymes produced considerable quantity mostly from aspergillus species and the penicillium species then like aspergillus oryzae, aspergillus niger then this pH tolerance is less than 3 that is their filamentous fungi is suitable for microbial solid state fermentation process specially because their morphology allow them to colonize and penetrate the solid surface.

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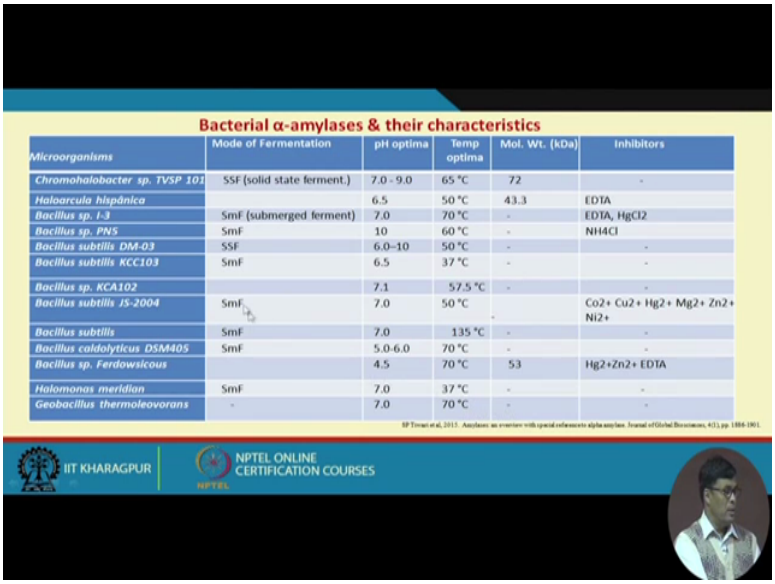


| Microorganisms | Mode of Fermentation | pH optima | Temp optima | Mol. Wt. (kDa) | Inhibitors |
|---|---|---|---|---|---|
| Chromohalobacter sp. TVSP 101 | SSF (solid state ferment.) | 7.0 - 9.0 | 65 °C | 72 | - |
| Haloarcula hispânica | | 6.5 | 50 °C | 43.3 | EDTA |
| Bacillus sp. I-3 | SmF (submerged ferment) | 7.0 | 70 °C | - | EDTA, HgCl2 |
| Bacillus sp. P N5 | SmF | 10 | 60 °C | - | NH4Cl |
| Bacillus subtilis DM-03 | SSF | 6.0–10 | 50 °C | - | - |
| Bacillus subtilis KCC103 | SmF | 6.5 | 37 °C | - | - |
| Bacillus sp. KCA102 | | 7.1 | 57.5 °C | - | - |
| Bacillus subtilis JS-2004 | SmF | 7.0 | 50 °C | - | Co2+ Cu2+ Hg2+ Mg2+ Zn2+ Ni2+ |
| Bacillus subtilis | SmF | 7.0 | 135 °C | - | - |
| Bacillus caldolyticus DSM405 | SmF | 5.0-6.0 | 70 °C | - | - |
| Bacillus sp. Ferdowsicous | | 4.5 | 70 °C | 53 | Hg2+Zn2+ EDTA |
| Halomonas meridian | SmF | 7.0 | 37 °C | - | - |
| Geobacillus thermoleovorans | - | 7.0 | 70 °C | - | - |

Now this gives you a table that indicate that that the what is the mode of fermentation what different types of microorganisms and what is optimum pH required for this fermentation process, what is the temperature required and what is the molecular weight of enzymes that we produce by this and what are the different inhibitor is is present. This bacterial alpha amylase that produce from this as different characteristics.

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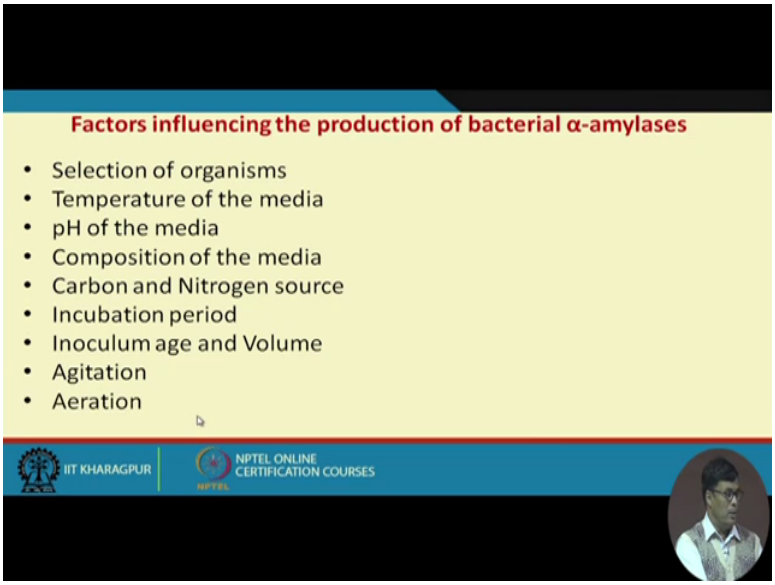


Now factors influencing the production of alpha amylase is the selection of microorganism that plays very important role I told you the industrial strain should higher productivity the temperature of the media plays very important role, pH of the media plays very important role. Composition of the media, carbon nitrogen source, incubation period, inoculum age and agitation and aeration these are the couple of things that plays very important role for alpha amylase production.

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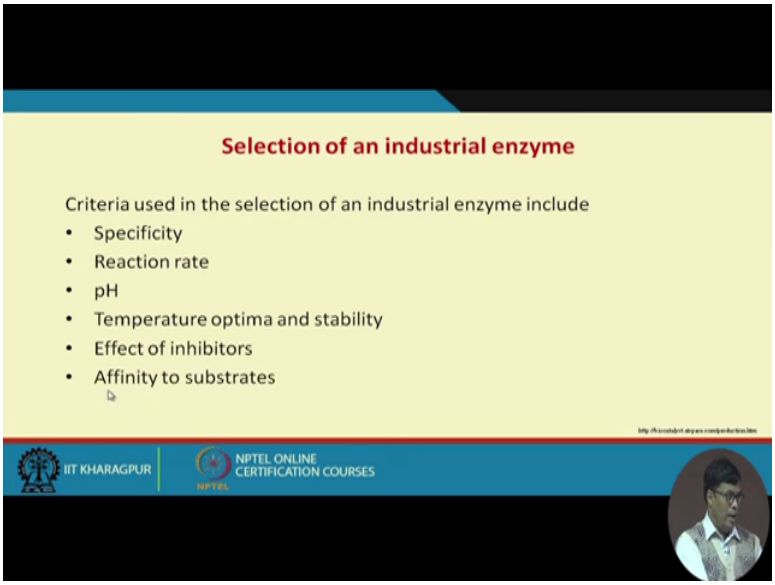


If you look at the selection for the industrial enzyme criteria is that that specificity and reaction rate and pH, temperature, optima and stability the stability of the enzymes plays very important role as per industry concerned and stability of the enzymes is usually determined

from the half-life of the enzymes so usually we prescribe the half-life of the enzymes should be as high as possible. Effect of inhibitors how inhibitors whether it is easily that affect that activity of the enzymes that is also very important affinity to substrate.

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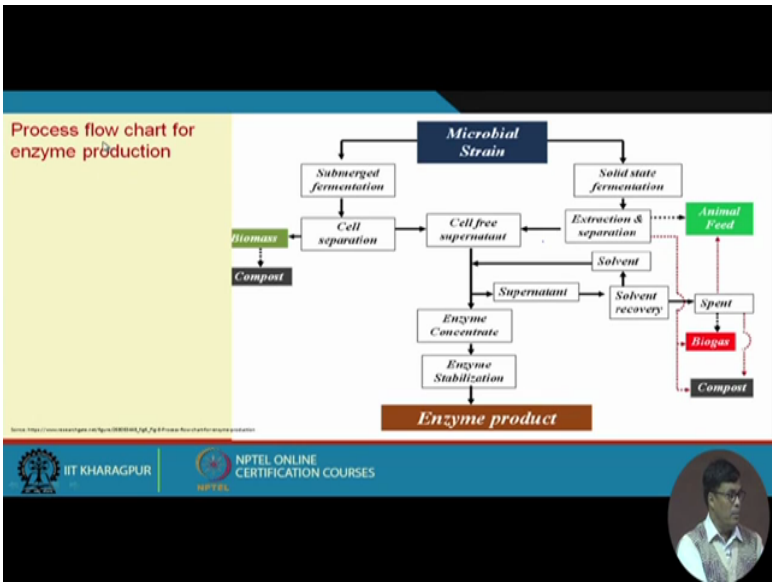


Now if you look at the process flow diagram for this enzyme production is here you have you have different microbial strength, we have submerged I I already explained you the submerge fermentation and solid state fermentation process. In submerged fermentation microorganism grow throughout the liquid and solid state fermentation that microorganism also grow throughout the solid media that this is usually in the liquid media.

Then this is the cells separation due take place. This is this is the biomass it can be used as a composed I showed I told you that this alpha amylase is the extra cellular product, it comes out of the cells so it present in the supernatant, you can you can make it concentrate and then you you stabilize by adding certain ingredients in it substrate analogue or some other material and then this product has usually in the we do the market similarly we do it from this solid state fermentation process.

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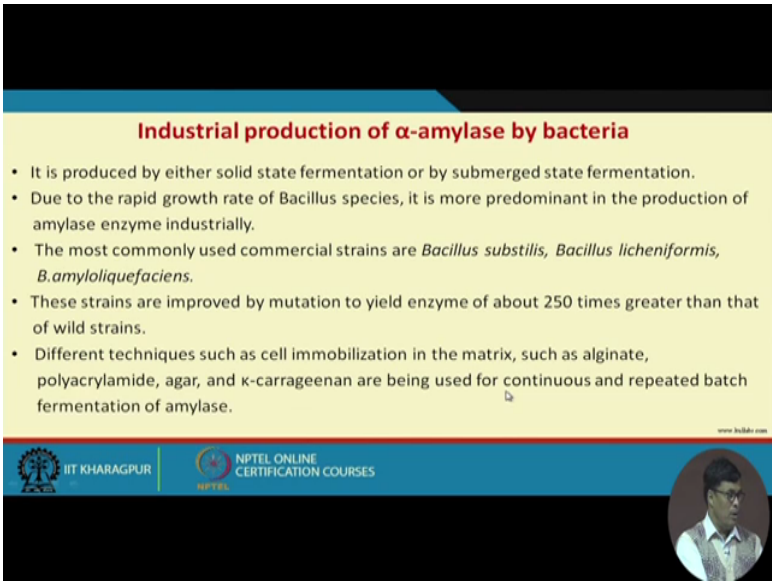


Now industrial industrial production of alpha amylases bacteria it is produced either solid state or by submerged fermentation process as I mentioned before and due to raid growth of bacillus species it is more predominant in the production of amylase in the industrial purpose then most commonly used commercial enzyme is bacillus subtilis and bacillus licheniformis and bacillus amylioliquefaciens, this is the mostly used by the industry.

These strains are improved by mutation to yield the enzyme about 250 times greater than the wild strain. Different techniques such as the cell immobilization in the matrix such as alginate polyacrylamide, agar and k-carrageenan are being used for continuous and rapid batch fermentation of amylase.

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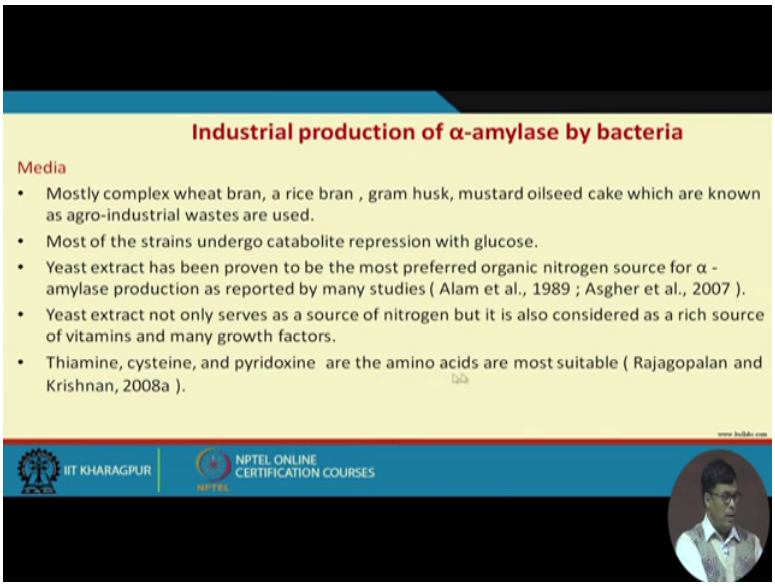

So this is for the degradation of starch we use that media used mostly the complex that wheat bran and rice bran, gram husk, mustard oilseed cake which known as a agriculture waste agricultural waste is used. Most of the strain undergo the catabolite repression with the glucose the glucose is the undesirable product in this because glucose gives some (())(16:09) effect

The yeast extracts has been proven to be the most preferred organic nitrogen source for alpha amylase production as reported by many studies, then yeast extract not only serve for the nitrogen source but also rich source of vitamin and other growth factor. The thiamine, cysteine and pyridoxine are the amino acids found mostly suitable for alpha amylase production.

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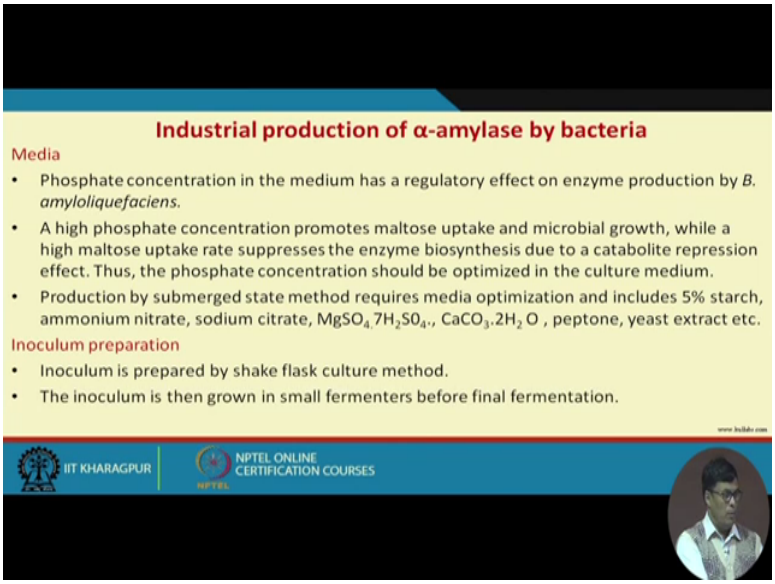


The media we have first paid concentration of the media as a regulated effect on the enzyme production by beta amyloliquefaceins, a high phosphate concentration promote the maltose uptake and the microbial growth while a high maltose uptake rate suppreses the enzyme biosynthesis due to the catabolite repression effect thus the phosphate concentration should be optimized in the cultivation broth.

The production by submerged state method required the medium optimization including 5 percent starch ammonium nitrate and sodium sodium citrate, magnesium sulphate, H2SO4 and and calcium calcium hydroxide 2H2O and then then yeast extract etc. Then inoculum preparation, inoculum is prepared in the safe flask then inoculum is grown in the small fermenter then finally in the production fermenter.

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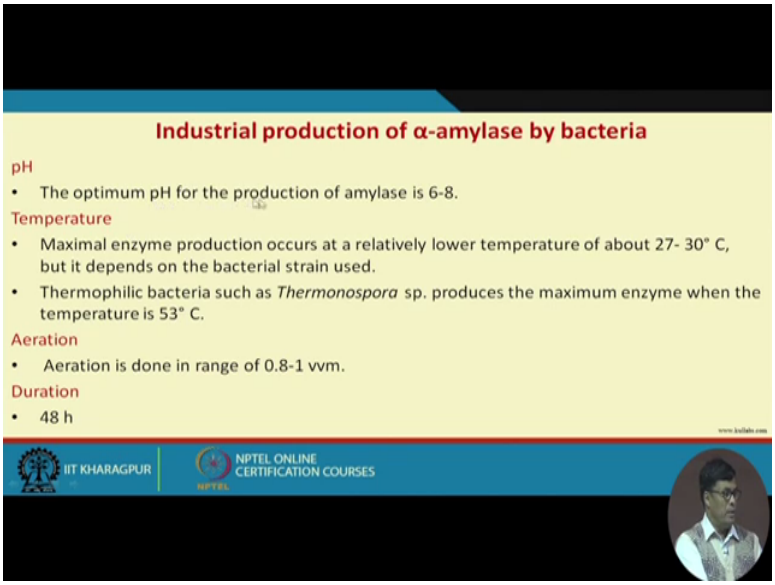


The optimum pH of the production is 6 6 to 8 and temperature maximum enzyme production occurs and relatively lower temperature 27 to 30 degree centigrade but it depends on the bacterial strain used. The thermophilic bacteria such as the thermonospora species produces maximum enzyme when the temperature is 53 degree centigrade, aeration is done in the range of 0.8 to 1 ppm vvm the duration of this fermentation is about 48 hours because this is a bacterial fermentation, this usually takes less time.

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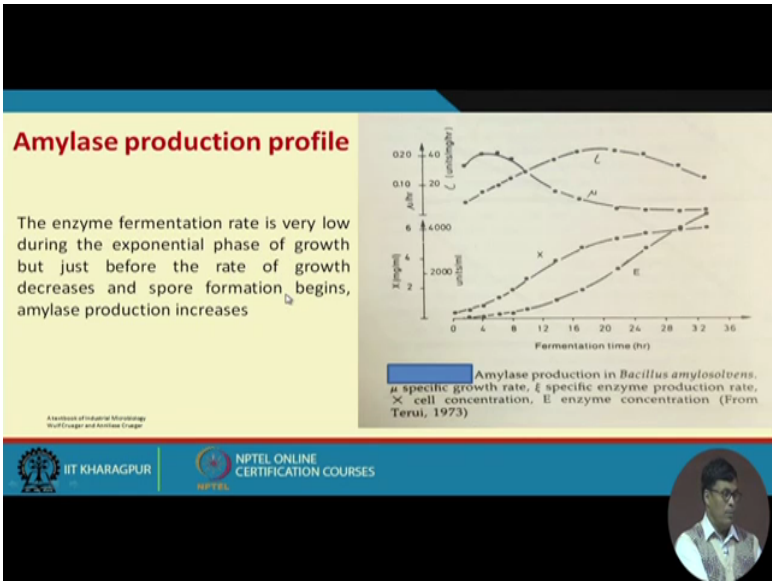


If you go for the fungal fermentation process it will take little longer time. The enzyme fermentation rate is slow during the exponential growth phase of the of the growth phase and just before the rate growth decreases the spore formation begins, amylase production

increases. So here you can visualize from here that enzyme this is the enzyme production this is the cell mass concentration that is that is cell mass that is growing like this. So when when the after sometimes when when that cell mass grows maximum and sporulation starts then enzyme production increases.

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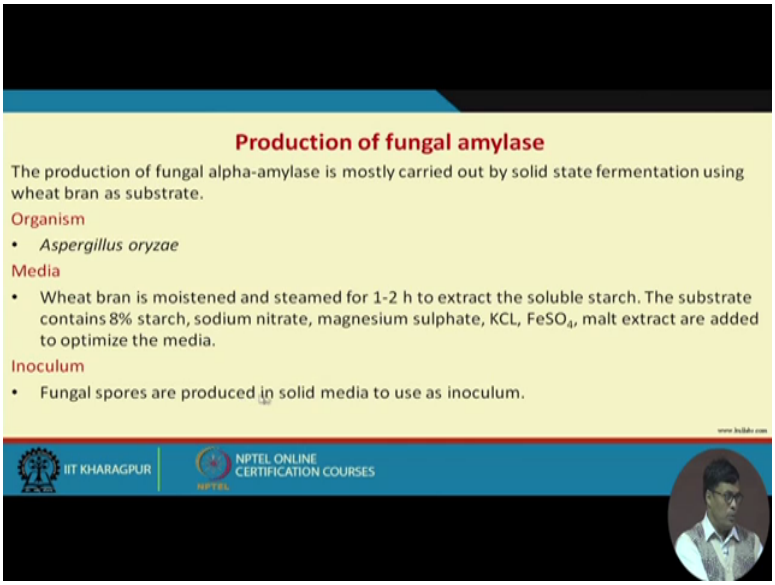


The production of fungal amylase similarly the particularly aspergillus oryzae is used mostly it is produced by using the solid state fermentation process, the wheat bran is moisture and steamed 1 to 2 hours to extract the soluble starch the substrate contains 8 percent starch and the and the sodium nitrate and magnesium sulphate KCL and ferrous sulphate malt extracts are added to optimise the media. Inoculum fungal spores are fungal I told you that fungal spores is used in the solid media to use as a inoculum.

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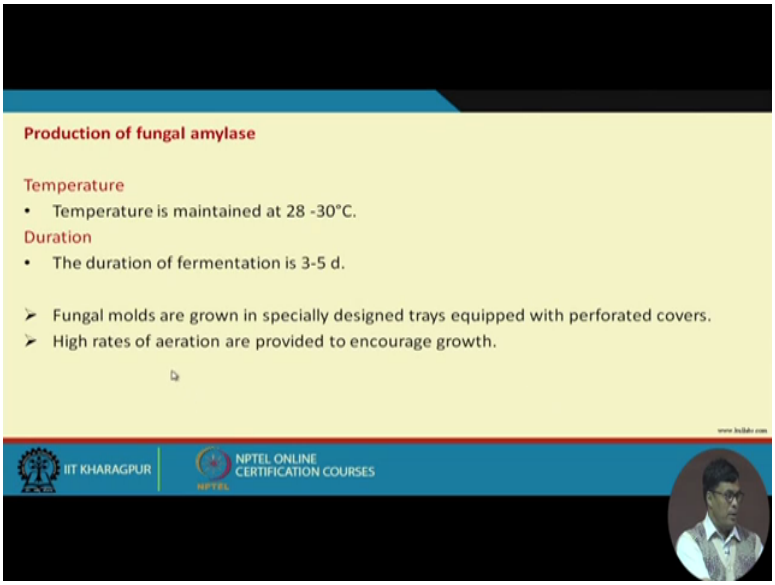


That production of fungal enzyme temperature is 28 to 30 degree centigrade, the duration time is 3 to 5 days I I told you in the case of bacterial fermentation it is 2 days so it is it takes quite long time 3 to 5 days. Fungal molds are grown specially designed trays equipped with perforated covers and high rate of aeration provided encourage the growth because this is the aerobic fermentation process.

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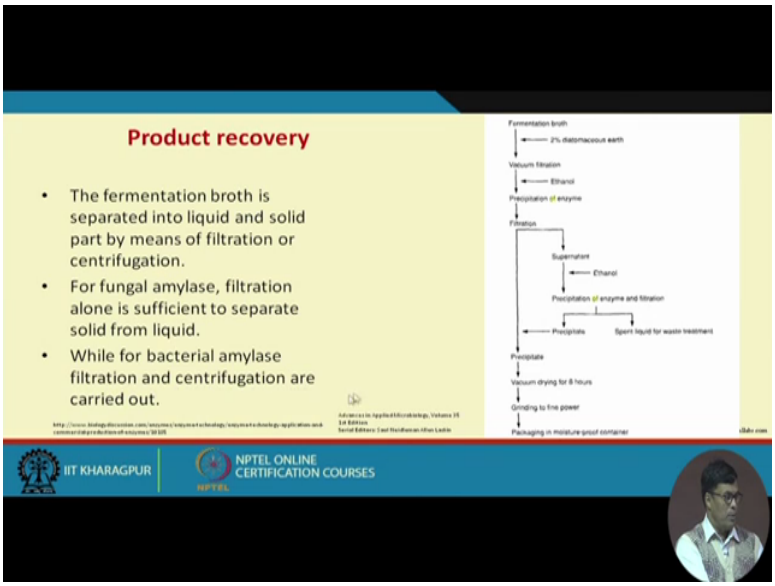


Now product recovery is like this the fermentation broth is separated into liquid and solid part by means of filtration and centrifugation. This is the fermentation broth we give some kind of we add some kind of 2 percent diatom this is kind of filtered aid then vacuum filtration then we we put ethanol and precipitation of enzyme takes place with the filtration supernatant we

discuss the precipitation with vacuum dry for 8 hours grinding in powder packaging moisture (())(21:03) we get the amylase and this is used for the waste treatment processes.

So this is for fungal amylase, filtration alone is sufficient to separate the solid from the liquid while the bacterium amylase filtration and centrifugation are carried out.

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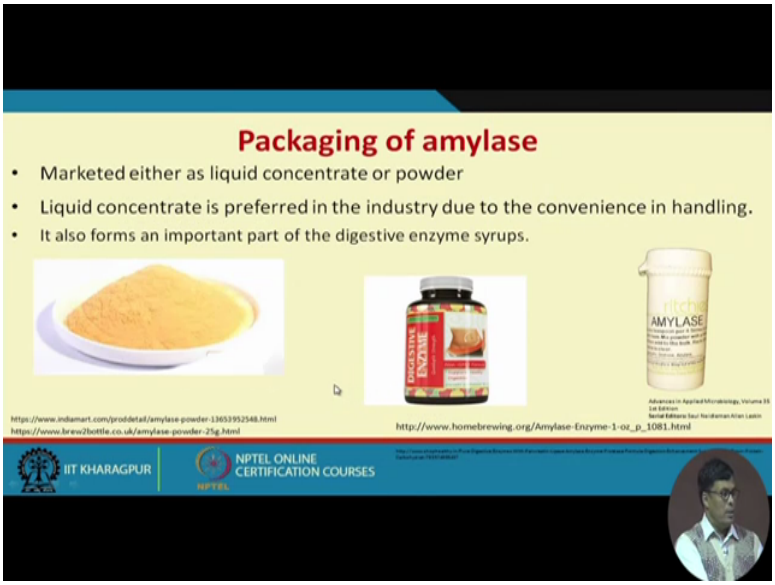


This is the you can you can see the product that has been market in the different forms. The marketed of amylase that either in the liquid concentrate or powder, liquid concentrate is preferred in the in the industry due to the convenience of handling because when you mix with this, the liquid can mix thoroughly but when you use solid then you have to (())(21:47) suspension so that is very important it is it also form an important part of digestive enzyme so you can see this this is powdered form and this is liquid form this again powder form that marketed.

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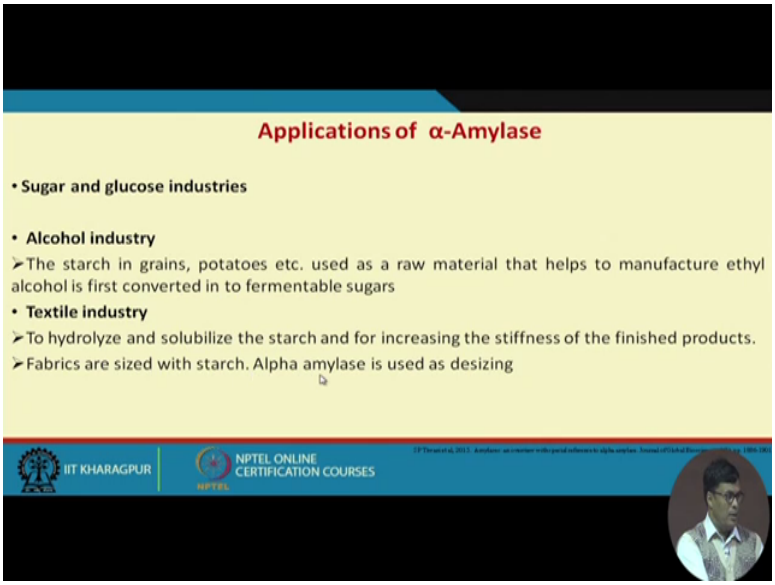


So the application of alpha amylase is tremendous the sugar and glucose industries largely use then we have used the alcohol industry I told you that IFB agro industry which is located close to Khadakpur that they produce alcohol from from broken rice which is used as a starchy, starch source and starch is a grain, potatoes etc is use as raw material to help the manufacture of ethanol is the first converted in the fermentable sugar.

Now textile industry to hydrolyse and soluble starch for for increasing the stiffness of the finished product so this is required and fabrics, fabric size is starched amylase is used for desizing purpose.

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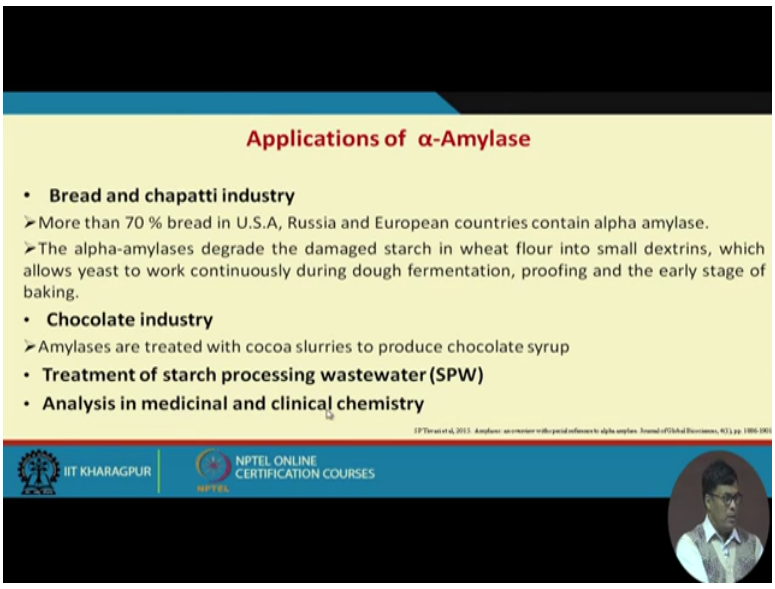

So application of their the bread and chapatti industry is largely used to make it a soft because you know that more than 70 percent bread USA USA, Russia and European county contains alpha amylase so that we can easily digest it and alpha amylases degrade the damaged starch of the wheat wheat flour into the small dextrins which allows the yeast to work continuously during the dough fermentation and proofing and early stage of baking.

The chocolate industry the amylase is treated with cocas slurries to produce the chocolate syrup. Treatment of starch processing waste water this analysis of medicinal and clinical chemistry it is largely used.

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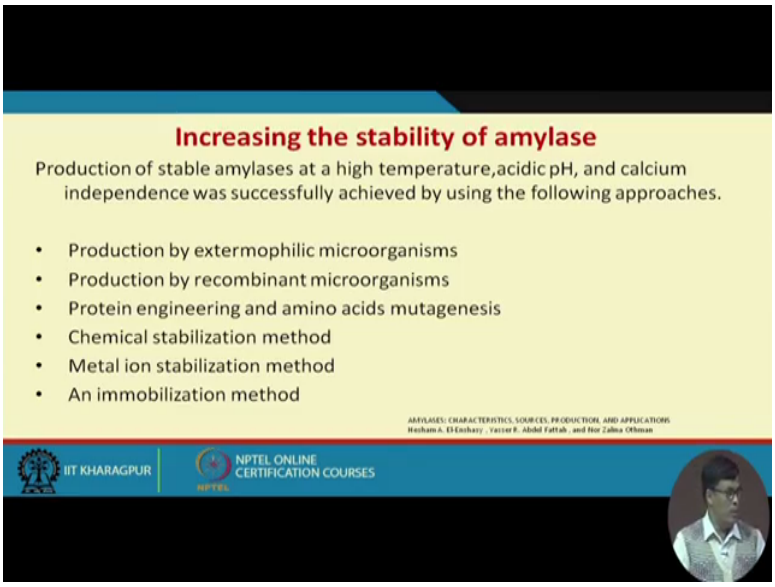


The increasing of stability of alpha amylase plays very important role their production of stable amylase is a high temperature I told you calcium plays very important important effect on that that that acidic pH and calcium independence are successfully achieved by using the following approach that production of the extermophilic organisms because if you use the extermophilic which can which can grow at a very high temperature and is expected this enzyme enzyme produced by this organism they are quite quite stable at high temperature from by using the recombinant enzyme.

Protein engineering amino acid mutagenesis and chemical stabilization method I told you by using some kind of substrate analogue or it is possible to increase the stability of the enzyme and metal ion stabilization methods and immobilization method largely used for the for the stability of the enzymes. So in this lecture I tried to discuss its (())(24:51) topic that is the

alpha amylase production, and which is largely used both in the food industry as well as in the pharmaceutical industry, thank you very much.