

Course on Industrial Biotechnology
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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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Amylase

- Among the industrially important enzymes, proteases and amylases are considered to be the most prominent enzymes since they are widely utilized in brewing, detergent, and food industries.
- After the World War II, enzyme applications increased due to advances in industrial microbiology and biochemical engineering.
- An amylase is an enzyme that catalyses the hydrolysis of starch into sugars.
- Amylase is present in the saliva of humans and some other mammals, where it begins the chemical process of digestion.

<https://en.wikipedia.org/wiki/Amylase>

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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 stain due to some fat type of material to use (02:13) that also do the it can remove the stain 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.

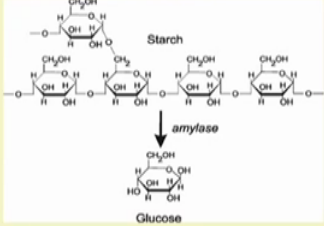
After the World War 2 the enzymes application increases due to the advances in industrial biotechnology and biochemical engineering and amylase is an enzyme that catalyse and hydrolyse the starch into sugar. And amylase is present in the saliva of human because we know our saliva contains the kind of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

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Types of amylase

All amylases are glycoside hydrolases. These enzymes are classified according to the manner in which the glycosidic bond is attacked as follows.

1. α -Amylase
2. β -Amylase
3. γ -Amylase



<https://www.biodid.com/resources/application-mechanisms/digestion-of-polysaccharides-part-1/>

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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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Types of amylase

α -Amylase

- α -amylases cleave α ,1-4 glycosidic bonds present in the inner part (endo) of the amylose or amylopectin chain of starch.
- Endo-amylase.
- End products of are oligosaccharides with varying length with α -configuration and α -limit dextrin.
- The α -amylases are calcium metalloenzymes, completely unable to function in the absence of calcium



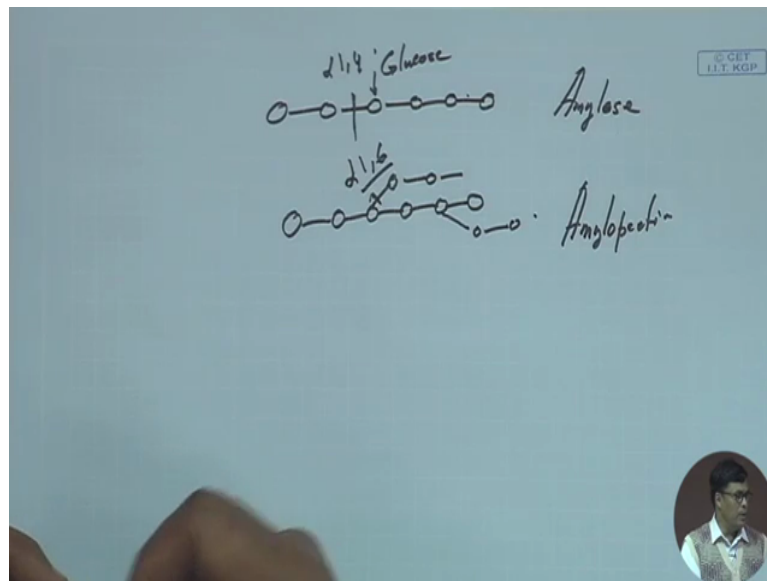
Human salivary amylase: calcium ion visible in pale khaki, chloride ion green

SP Thirumal et al., 2011. Amylase: an overview with special reference to alpha amylase. Journal of Global Research, 4(3), pp. 186-190

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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 amylose and the other is called amylopectin.

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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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Types of amylase

- α -amylases are often divided into two categories according to the degree of hydrolysis of the substrate.

α -amylases

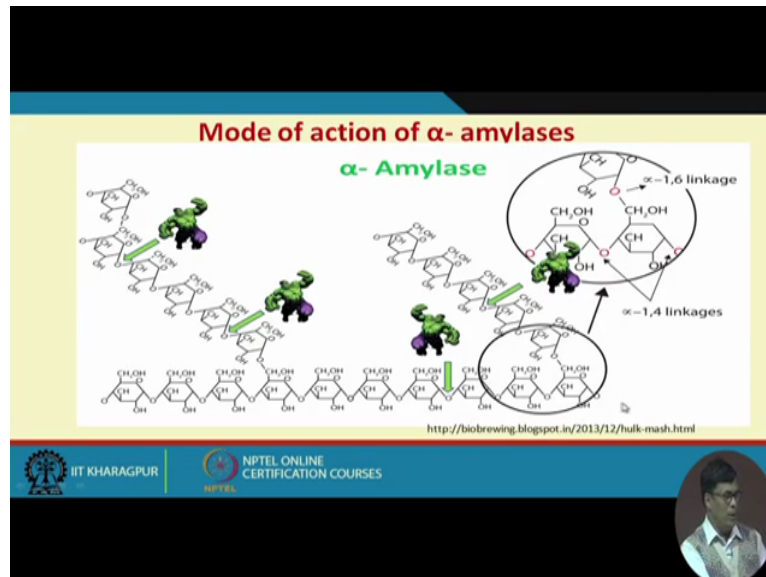
- Saccharifying α -amylases**
 - They hydrolyze 50 to 60% of the glycosidic linkages of starch
- Liquefying α -amylases**
 - Liquefying α -amylases cleave about 30 to 40% of the glycosidic linkages of starch.

BP Tripathi et al. 2011. Amylases as enzymes with special reference to alpha-amylase. Journal of Global Biochemistry, 4(1), pp. 1888-1901

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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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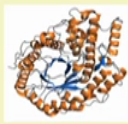
There is the two types of alpha amylases and this is they call endo endo endoamylase endoamylase means that this can randomly attack the starch molecules at any position, you see that any position here here here at the so in process we produce oligosaccharide oligosaccharide means it is dimer, trimer, 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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Types of amylase


β - Amylase

- β -amylase also works on α -1,4-glycosidic bonds, but only on the non-reducing end of the polysaccharide chain, making it slower, cleaving two glucoses off at a time resulting in a single maltose molecule.
- This enzyme acts on starch, glycogen and related polysaccharides and oligosaccharides producing beta-maltose by an inversion.
- Beta-amylase is found in bacteria, fungi, and plants
- Bacteria and cereal sources are the most heat stable.
- The optimum pH for β -amylase is 4.0–5.0



<https://en.wikipedia.org/wiki/Beta-amylase> TP Desai et al, 2011. Amylase: an overview with special reference to alpha amylase. Journal of Global Biosciences, 03, pp. 189-190

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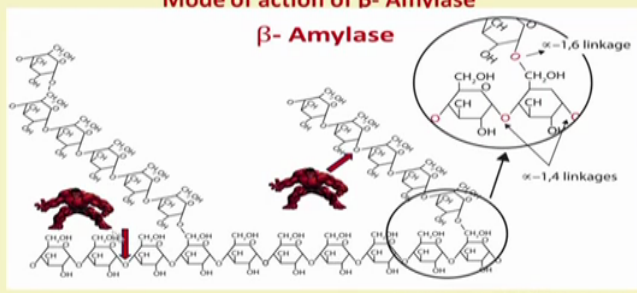


And then we have other types of amylase, the beta amylase the beta amylase are they walk alpha 1-4 glycosidic bond but only on the non-hydrogen non reducing end of the polysaccharide and making it slow and cleave two glucose off at a time resulting a single maltose molecule. So I can show you this it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

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Mode of action of β - Amylase

β - Amylase




α -1,6 linkage

α -1,4 linkages

<http://biobrewing.blogspot.in/2013/12/hulk-mash.html>

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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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Types of amylase

γ -Amylase

- γ -amylase cleaves $\alpha(1-6)$ glycosidic linkages, in addition to cleaving the last $\alpha(1-4)$ glycosidic linkages at the non-reducing end of amylose and amylopectin, yielding glucose.
- γ -amylase is most efficient in acidic environments and has an optimum pH of 3.

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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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History of amylase production

- The history of amylases began in 1811 when the first starch degrading enzyme was discovered by Kirchhoff in wheat.
- The α -amylases were named by Kuhn in 1925, because the hydrolysis products are in the alpha configuration.
- Amylase was the first enzyme to be discovered and isolated by Anselme Payen, 1833.
- Interestingly, the first enzyme produced industrially was an amylase from a fungal source in 1894, which was used as a pharmaceutical aid for the treatment of digestive disorders.
- Boidin & Effront, 1917 were the first to use *Bacillus subtilis* and *Bacillus mesentericus* for the production of α -amylases on commercial scale using large fermentors in submerged fermentation.

© P. Tharan et al., 2015. Amylase: an overview with special reference to alpha amylase. Journal of Global Biotechnol, 4(1), pp. 1896-1903

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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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Sources of α -Amylase

Plant sources

- They play crucial roles in plant growth regulation.
- β -amylase appears prior to α -amylase to initiate the germination process.
- Plant sources had not been considered with enough significance as the source of these enzymes because of low productivity and difficulty in cultivation.

Animal sources

- Ptyalin, an α -amylase (α -1,4- α -D-glucan-4-glucanohydrolase; E.C. 3.2.1.1) is one of the most important enzymes in saliva.
- The pancreas and salivary gland make amylase (alpha amylase) to hydrolyze dietary starch into disaccharides and trisaccharides.

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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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Sources of α -Amylase

Amylases from microbial sources (both bacteria and fungi) are used for the industrial production due to following advantages.

- Cost effectiveness
- Consistency
- Less production time
- Ease of process modification and optimization.

Bacterial amylases

- *B. subtilis*, *B. stearothermophilus*, *B. licheniformis* and *B. amyloliquefaciens* are known to be good producers of α -amylase
- Thermostable enzymes isolated from thermophilic bacteria have commercial applications due to stability.

SP Dey et al, 2011. Amylase: an overview with special reference to alpha amylase. Journal of Global Biochemistry, 4(3), pp. 189-193

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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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Sources of α -Amylase

Bacterial enzymes

- They are exploited commercially due to their rapid growth rate leading to short fermentation cycles, capacity to secrete proteins into the extracellular medium and safe handling.

Fungal amylases

- Fungi produce considerable quantities of enzymes mostly from *Aspergillus* sp. and *Penicillium* sp. e.g. Filamentous fungi such as *Aspergillus oryzae* and *Aspergillus niger*
- α -amylase produced by *Aspergillus niger* has acid tolerance (pH < 3) and prevents bacterial contamination.
- Filamentous fungi are suitable microorganisms for solid state fermentation (SSF), especially because their morphology allows them to colonize and penetrate the solid substrate.

SP Dey et al, 2011. Amylase: an overview with special reference to alpha amylase. Journal of Global Biochemistry, 4(3), pp. 189-193

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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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Bacterial α -amylases & their characteristics

Microorganisms	Mode of Fermentation	pH optima	Temp optima	Mol. Wt. (kDa)	Inhibitors
<i>Chromohalobacter</i> sp. TVSP 101	SSF (solid state ferment.)	7.0 - 9.0	65 °C	72	-
<i>Halorarcula hispánica</i>	-	6.5	50 °C	43.3	EDTA
<i>Bacillus</i> sp. I-3	Smf (submerged ferment)	7.0	70 °C	-	EDTA, HgCl ₂
<i>Bacillus</i> sp. PNS	Smf	10	60 °C	-	NH ₄ Cl
<i>Bacillus subtilis</i> DM-03	SSF	6.0-10	50 °C	-	-
<i>Bacillus subtilis</i> KCC103	Smf	6.5	37 °C	-	-
<i>Bacillus</i> sp. KCA102	-	7.1	57.5 °C	-	-
<i>Bacillus subtilis</i> JS-2004	Smf	7.0	50 °C	-	Co ²⁺ Cu ²⁺ Hg ²⁺ Mg ²⁺ Zn ²⁺ Ni ²⁺
<i>Bacillus subtilis</i>	Smf	7.0	135 °C	-	-
<i>Bacillus caldolyticus</i> DSM405	Smf	5.0-6.0	70 °C	-	-
<i>Bacillus</i> sp. Ferdowsicus	-	4.5	70 °C	53	Hg ²⁺ +Zn ²⁺ + EDTA
<i>Halomonas meridian</i>	Smf	7.0	37 °C	-	-
<i>Geobacillus thermoleovorans</i>	-	7.0	70 °C	-	-

SP Tripathi et al., 2011. Amylases: an overview with special reference to alpha amylase. Journal of Global Biotechnology, 4(1), pp. 138-140.

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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Factors influencing the production of bacterial α -amylases

- Selection of organisms
- Temperature of the media
- pH of the media
- Composition of the media
- Carbon and Nitrogen source
- Incubation period
- Inoculum age and Volume
- Agitation
- Aeration

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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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Selection of an industrial enzyme

Criteria used in the selection of an industrial enzyme include

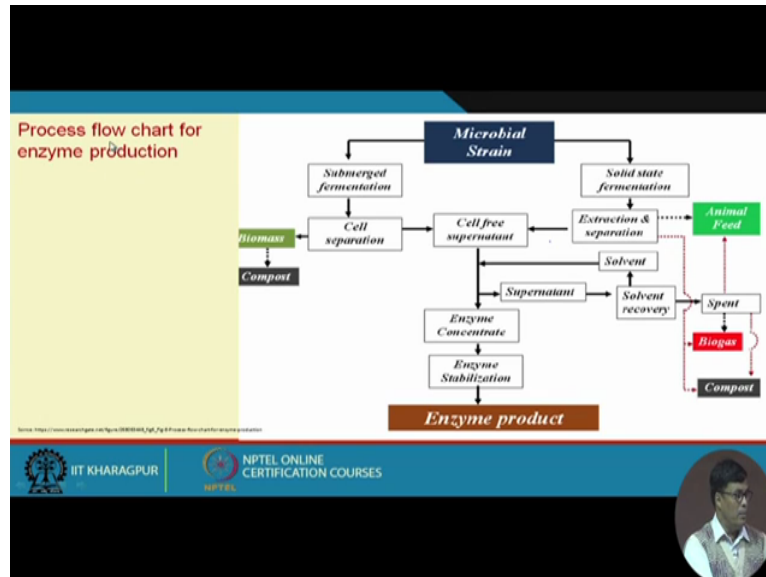
- Specificity
- Reaction rate
- pH
- Temperature optima and stability
- Effect of inhibitors
- Affinity to substrates

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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.

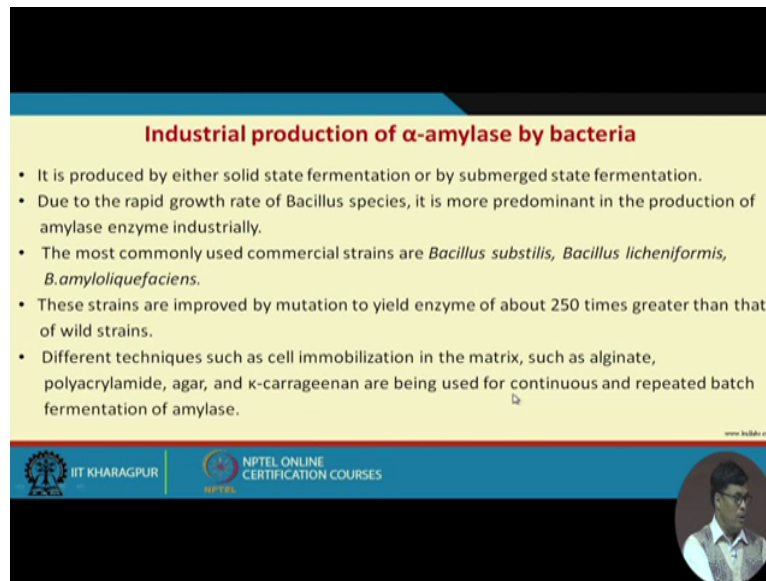
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Now if you look at the process flow diagram for this enzyme production is here you have you have different microbial strength, we have submerged I I already explained you the submerge fermentation and solid state fermentation process. In submerged 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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Industrial production of α -amylase by bacteria

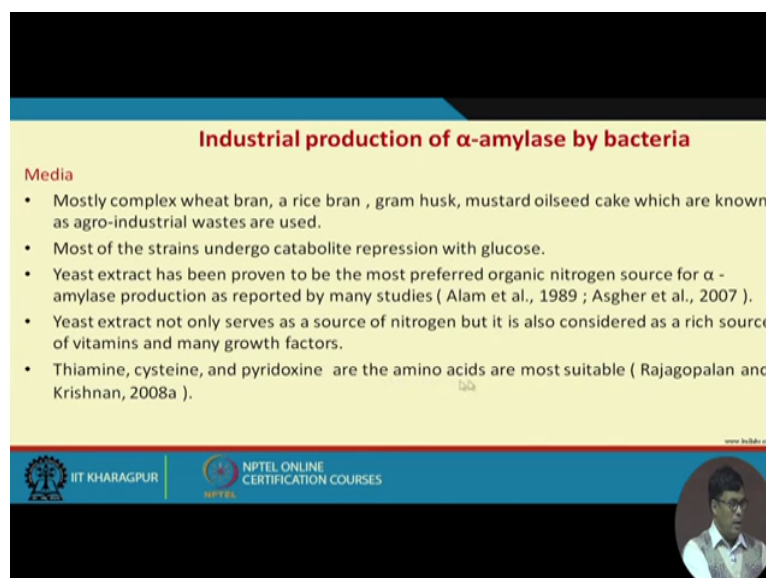
- It is produced by either solid state fermentation or by submerged state fermentation.
- Due to the rapid growth rate of *Bacillus* species, it is more predominant in the production of amylase enzyme industrially.
- The most commonly used commercial strains are *Bacillus subtilis*, *Bacillus licheniformis*, *B. amyloliquefaciens*.
- These strains are improved by mutation to yield enzyme of about 250 times greater than that of wild strains.
- Different techniques such as cell immobilization in the matrix, such as alginate, polyacrylamide, agar, and κ -carrageenan are being used for continuous and repeated batch fermentation of amylase.

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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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Industrial production of α -amylase by bacteria

Media

- Mostly complex wheat bran, a rice bran , gram husk, mustard oilseed cake which are known as agro-industrial wastes are used.
- Most of the strains undergo catabolite repression with glucose.
- Yeast extract has been proven to be the most preferred organic nitrogen source for α - amylase production as reported by many studies (Alam et al., 1989 ; Asgher et al., 2007).
- Yeast extract not only serves as a source of nitrogen but it is also considered as a rich source of vitamins and many growth factors.
- Thiamine, cysteine, and pyridoxine are the amino acids are most suitable (Rajagopalan and Krishnan, 2008a).

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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 (Catabolite)(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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Industrial production of α -amylase by bacteria

Media

- Phosphate concentration in the medium has a regulatory effect on enzyme production by *B. amyloliquefaciens*.
- A high phosphate concentration promotes maltose uptake and microbial growth, while a high maltose uptake rate suppresses the enzyme biosynthesis due to a catabolite repression effect. Thus, the phosphate concentration should be optimized in the culture medium.
- Production by submerged state method requires media optimization and includes 5% starch, ammonium nitrate, sodium citrate, $MgSO_4 \cdot 7H_2O$, $CaCO_3 \cdot 2H_2O$, peptone, yeast extract etc.

Inoculum preparation

- Inoculum is prepared by shake flask culture method.
- The inoculum is then grown in small fermenters before final fermentation.

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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 suppresses 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, H_2SO_4 and and calcium calcium hydroxide $2H_2O$ 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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Industrial production of α -amylase by bacteria

pH

- The optimum pH for the production of amylase is 6-8.

Temperature

- Maximal enzyme production occurs at a relatively lower temperature of about 27- 30° C, but it depends on the bacterial strain used.
- Thermophilic bacteria such as *Thermonospora* sp. produces the maximum enzyme when the temperature is 53° C.

Aeration

- Aeration is done in range of 0.8-1 vvm.

Duration

- 48 h

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The optimum pH of the production is 6 to 8 and temperature maximum enzyme production occurs at a 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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Amylase production profile

The enzyme fermentation rate is very low during the exponential phase of growth but just before the rate of growth decreases and spore formation begins, amylase production increases

Amylase production in *Bacillus amylosolvans*.
 μ specific growth rate, ξ specific enzyme production rate, \times cell concentration, E enzyme concentration (From Terui, 1973)

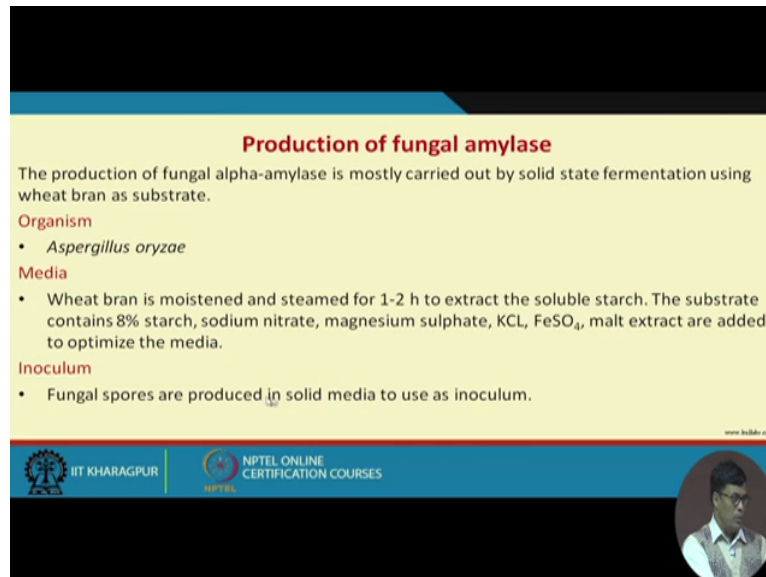
Fermentation time (hr)	μ (hr ⁻¹)	Cell concentration (x 10 ⁸ spores/ml)	Enzyme concentration (units/ml)
0	0.00	0	0
4	0.18	1000	0
8	0.18	2000	0
12	0.18	3000	0
16	0.18	4000	0
20	0.18	4000	0
24	0.18	4000	0
28	0.18	4000	0
32	0.18	4000	0
36	0.18	4000	0

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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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Production of fungal amylase

The production of fungal alpha-amylase is mostly carried out by solid state fermentation using wheat bran as substrate.

Organism

- *Aspergillus oryzae*

Media


- Wheat bran is moistened and steamed for 1-2 h to extract the soluble starch. The substrate contains 8% starch, sodium nitrate, magnesium sulphate, KCL, FeSO₄, malt extract are added to optimize the media.

Inoculum

- Fungal spores are produced in solid media to use as inoculum.

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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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Production of fungal amylase

Temperature

- Temperature is maintained at 28 -30°C.

Duration

- The duration of fermentation is 3-5 d.

➤ Fungal molds are grown in specially designed trays equipped with perforated covers.

➤ High rates of aeration are provided to encourage growth.

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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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Product recovery

- The fermentation broth is separated into liquid and solid part by means of filtration or centrifugation.
- For fungal amylase, filtration alone is sufficient to separate solid from liquid.
- While for bacterial amylase filtration and centrifugation are carried out.

Advances in Applied Microbiology, Volume 18
1st Edition
Second Edition: 2nd and Third Editions: John Leslie

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Product recovery flowchart:

```
graph TD
    A[Fermentation broth] --> B[2% diatomaceous earth]
    B --> C[Vacuum filtration]
    C --> D[Ethanol]
    C --> E[Precipitation of enzyme]
    E --> F[Filtration]
    F --> G[Supernatant]
    F --> H[Precipitation of enzyme and filtration]
    G --> I[Ethanol]
    H --> J[Spent liquid for waste treatment]
    H --> K[Precipitate]
    K --> L[Vacuum drying for 8 hours]
    L --> M[Grinding to fine powder]
    M --> N[Packaging in moisture-proof container]
```

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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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Packaging of amylase

- Marketed either as liquid concentrate or powder
- Liquid concentrate is preferred in the industry due to the convenience in handling.
- It also forms an important part of the digestive enzyme syrups.

The slide features three images illustrating different packaging forms of amylase: a white plate with a mound of yellow powder, a glass bottle with a red label, and a white plastic jar with a yellow label. Below the images are three URLs: <https://www.indiamart.com/prodetail/amylase-powder-13051952548.html>, <https://www.brew2bottle.co.uk/amylase-powder-25g.html>, and http://www.homebrewing.org/Amylase-Enzyme-1-or_p_1081.html. The slide also includes logos for IIT KHARAGPUR and NPTEL ONLINE CERTIFICATION COURSES, and a small portrait of a man in the bottom right corner.

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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Applications of α -Amylase

- **Sugar and glucose industries**
- **Alcohol industry**
 - The starch in grains, potatoes etc. used as a raw material that helps to manufacture ethyl alcohol is first converted in to fermentable sugars
- **Textile industry**
 - To hydrolyze and solubilize the starch and for increasing the stiffness of the finished products.
 - Fabrics are sized with starch. Alpha amylase is used as desizing

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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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Applications of α -Amylase

- **Bread and chapatti industry**
 - More than 70 % bread in U.S.A, Russia and European countries contain alpha amylase.
 - The alpha-amylases degrade the damaged starch in wheat flour into small dextrans, which allows yeast to work continuously during dough fermentation, proofing and the early stage of baking.
- **Chocolate industry**
 - Amylases are treated with cocoa slurries to produce chocolate syrup
- **Treatment of starch processing wastewater (SPW)**
- **Analysis in medicinal and clinical chemistry**

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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 dextrans 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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Increasing the stability of amylase

Production of stable amylases at a high temperature, acidic pH, and calcium independence was successfully achieved by using the following approaches.

- Production by extremophilic microorganisms
- Production by recombinant microorganisms
- Protein engineering and amino acids mutagenesis
- Chemical stabilization method
- Metal ion stabilization method
- An immobilization method

AMYLASES: CHARACTERISTICS, SOURCES, PRODUCTION, AND APPLICATIONS
Hebah A. El-Eshary, Yasser E. Abdel Fattah, and Hani Zahra Othman

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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 extremophilic organisms because if you use the extremophilic 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.