Industrial Biotechnology.
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Lecture-42.
Streptomycin Production.

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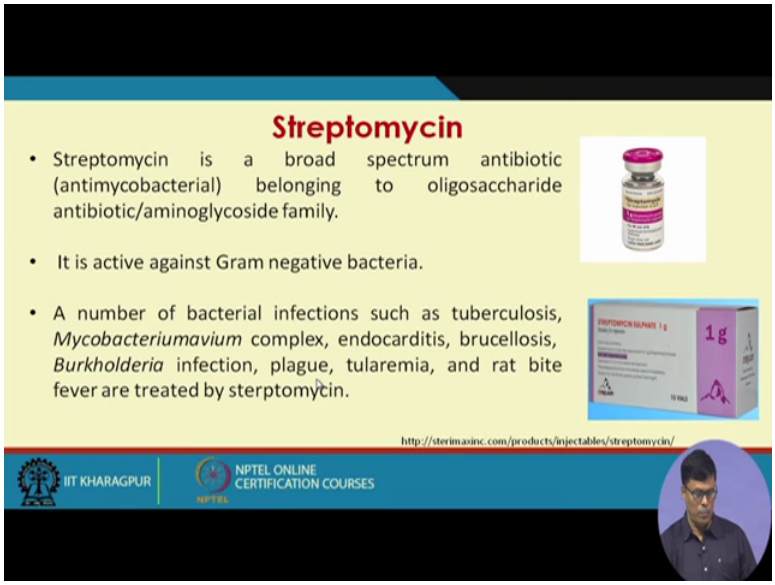

Welcome back to my course that Industrial Biotechnology. Now I think in the last two classes couple of classes we discussed the penicillin and cephalosporin fermentation process, now this lecture I want to tell something different though it is antibiotics, but this antibiotics streptomycin this is produced through the bacterial fermentation process. So let us give the introduction to streptomycin it is a broad spectrum antibiotics and belonging to the what you call amino glycoside family this is very important.

This is aminoglycoside family we know that kanamycin also include in this gentamycin kanamycin that include on this particular in this particular category. Then it is active against the gram negative bacteria and a number of bacterial infection such as the tuberculosis, mycobacteriumavium complex, endocarditis and brucellosis, the different plague and different rat bites fever are treated by streptomycin.

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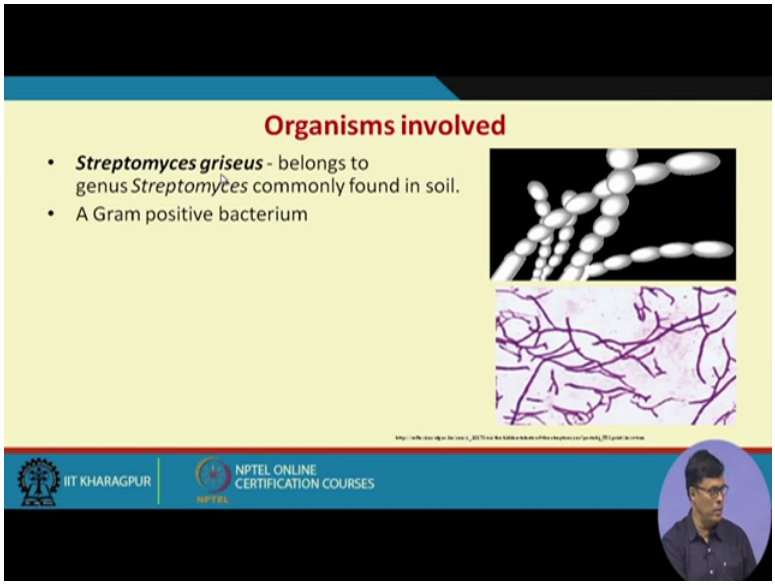

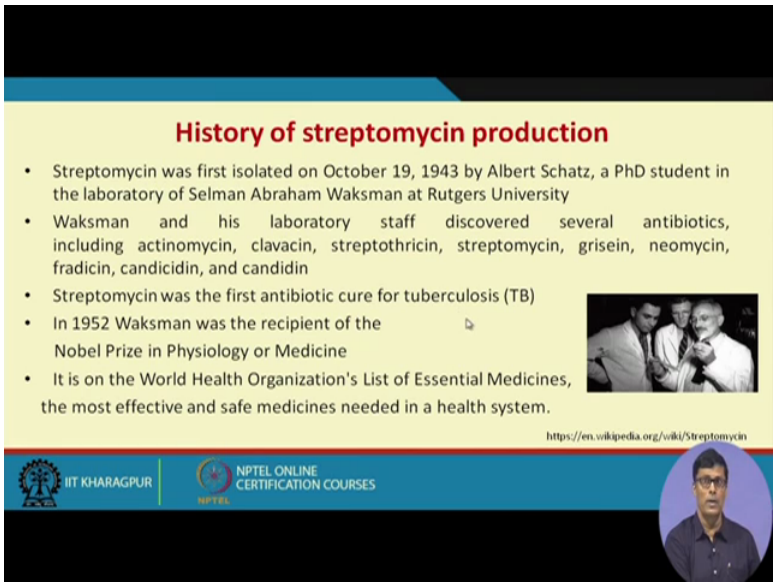

The streptomycin since it is a broad spectrum antibiotics it can be used for killing the different germs. Now if you look at the organisms that streptomycin griseus this is used for the production of streptomycin and this is commonly found in the soil and it is a gram positive bacteria. The streptomycin griseus strains are well known producer of antibiotics and significantly it is the secondary metabolites. I told you that your penicillin also secondary metabolites also cephalosporin also considered as a secondary metabolites.

They produce grey spores masses and grey yellow reverse pigment when they grow as colony. They grow in a wide pH 5 to 11, this is the speciality of this organism and this is how they look under the microscope. If you look at the history of streptomycin it has first isolated

October 19, 1943 by Albert Schatz, a PhD student laboratory in Selman Abraham Waksman at Rutgers University USA.

And then Waksman and his laboratory staff discovered the several antibiotics including actiomycin, clavacin, streptothricin and streptomycin, grisein, neomycin and different types of antibiotics they discovered. Streptomycin was the first antibiotics cured the tuberculosis. We know that TB is a deadly disease and and if you look at the history lot lots of you know well known people they were killed by due to that TB infection.

So in 1952 Waksman was the recipient of the Nobel Price in Physiology or Medicine, it is on the World Health Organisation List of Essential Medicine and the most effective and safe medicine needed in the health system. So this is the speciality of the streptomycin this is very effective against the tuberculosis and at WHO they consider this is a very safe medicine for this.

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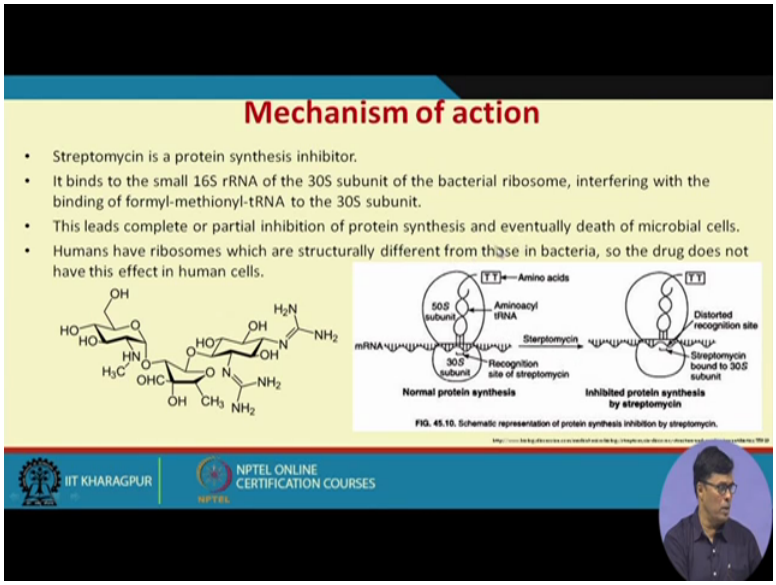

Now let us see that the how this antibiotic works that the streptomycin is a protein synthesis inhibitor that essential protein that require for the growth of the bacteria that synthesis they inhibit. And how it inhibits because it binds with the small 16S rRNA of the 30S subunit of the bacterial ribosomes interfering with the binding of formyl methionyl tRNA to 30S subunit.

This is how they are binding here and and this lead to complete or partially inhibit the protein synthesis eventually that death of the cells. Humans have the ribosomes which are structurally different from those in bacteria, so drug does not have this effect in the human

cells. So human cells that this does not have this effect this this kind of biochemical activity that we do not have the human system but this is only applicable to the bacterial system they disturb the essential some protein synthesis and they kill the microorganisms.

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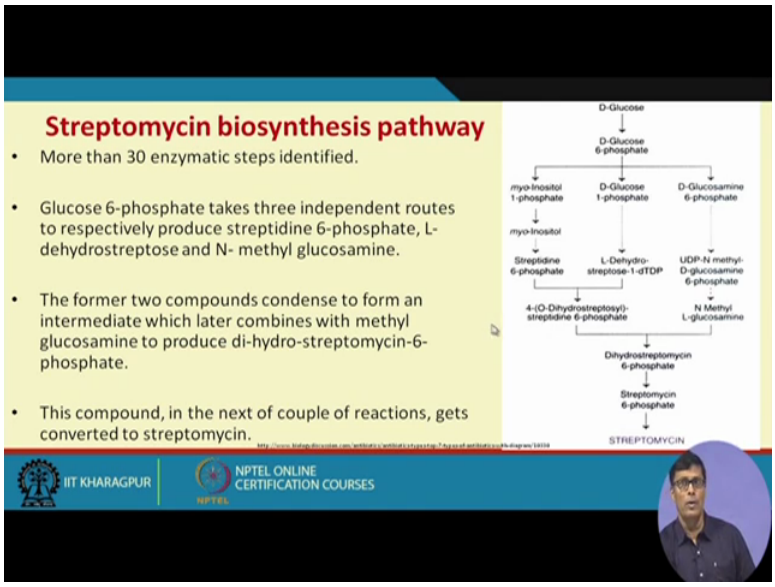

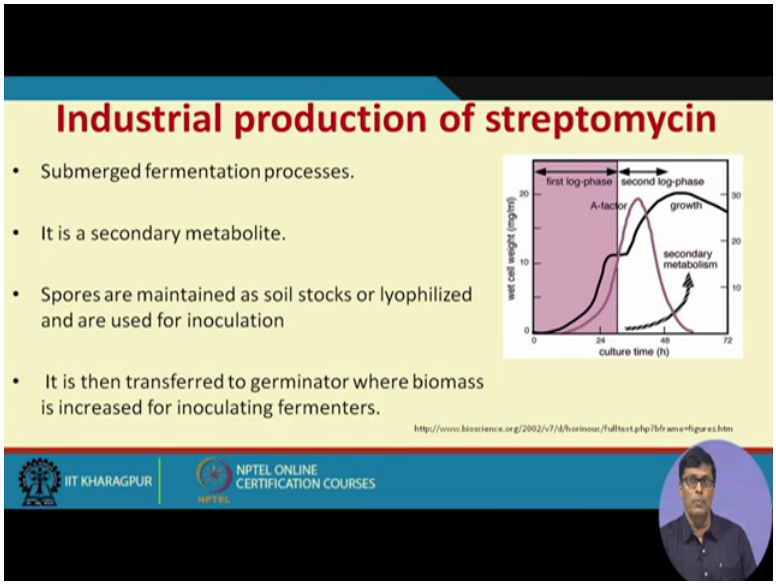

Now if you look at the biosynthesis pathway the glucose it produce the glucose6 phosphate then it produce that isositol this 1-phosphate and then D glucose 1-phosphate to glucosamine 6 phosphate then myo-inositol this all when they come when they combine and ultimately they form the dehydrostreptomycin 6 phosphate and then it comes finally the streptomycin production.

Now this streptomycin require production required 30 enzymatic steps because the several steps are required before it produce streptomycin. Glucose 6 phosphate takes 3 independent

routes to respectively produce the streptidine 6 phosphate and L- dehydrostreptose and N-methyl glucosamine. The former two compounds condense to form a intermediate which layer combines with methyl glucosamine to produce di-hydro-streptomycin 6 phosphate and this compound in the next couple of reaction gets converted to streptomycin.

This is how the streptomycin is produced biochemically. Now industrial production of streptomycin is like this it is produced through the submerged fermentation process, I I already explained in case of penicillin fermentation process that we use two type of fermenter, not only penicillin in citric acid also mostly I discussed in citric acid fermentation process, that it is usually produced both by 20 per cent citric acid is produced by surface fermentation and 80 per cent by submerged fermentation process.

And mostly we use in the fermentation unit submerge fermentation the reason is that that whole fermentation broth we get the, your microorganism will grow and give the product and that is why it is very effective. So submerged fermentation is very is used here, it is the secondary metabolites and spores are maintained as soil stocks or lyophilized and are used as for inoculations and this is then transferred to germinator where the biomass is increased for inoculating the fermenters.

So here also it is same as we shall have to produce the inoculum before we we transfer we use this in the production fermenter. And this is like this you have first lock phase, you have you can dioxin type of growth you can see it here that it grows diauxic growth we know that dioxin growth means in the, it is possible when your organism consume one particular carbon source and then when it is exhausted then only then then the other carbon source will be utilized then it is called dioxin.

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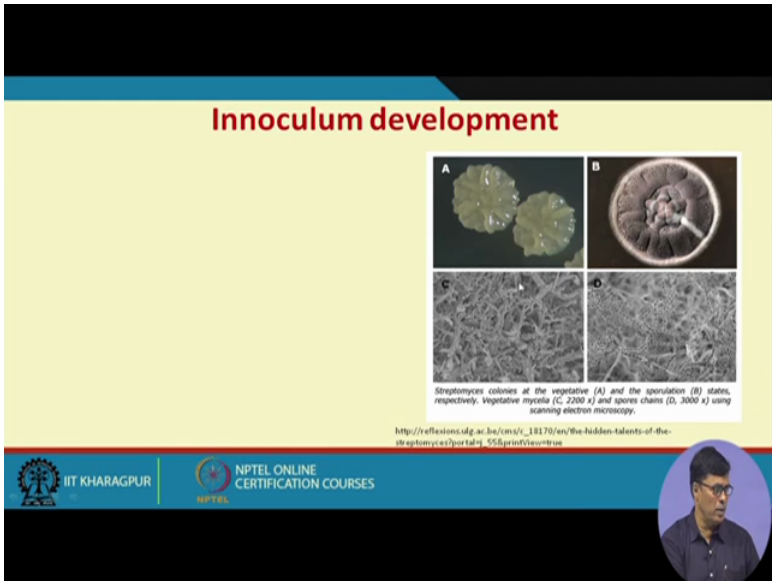

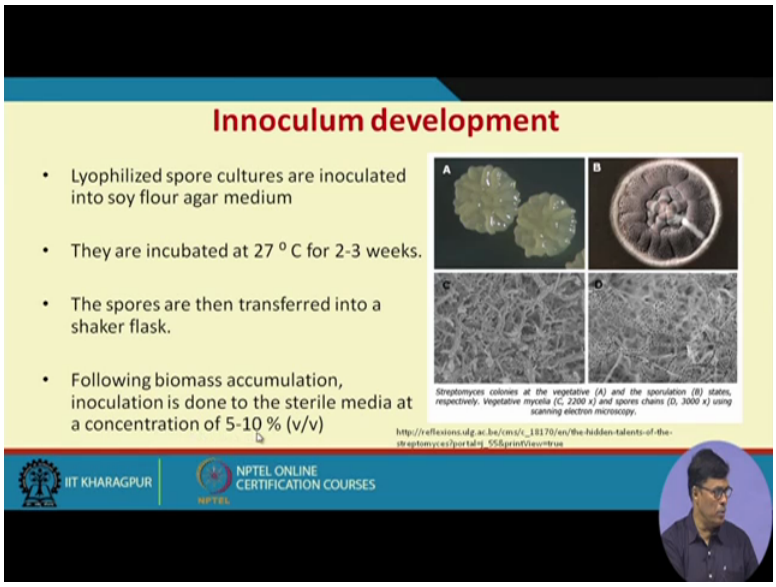

Usually it has been found that streptomycin fermentation process is follow the dioxin growth pattern. Now this under the microscope it is very interesting it has some resemblance with the fungal fermentation, though it is the bacterial fermentation process, but it has some resemblance, because you see in case of vegetative cells they have lot of branches we can see it is the what we have in case of fungal fermentation process, the bacterial fermentation this streptomycin griseus also have this kind of lot of branches and also it has this kind of small formation that is quite common in case of fungal fermentation process.

So lyophilized spore cultures are inoculated into soy flour agar medium and they are inoculated at 27 degree centigrade for 2 to 3 weeks, the spores are then transferred into shaker flask and following the biomass accumulation and inoculation is done in a sterile

media a concentration 5 to 10 per cent. I told you whenever we use any kind of inoculum to the production fermenter we always use 5 to 10 per cent of the volume by volume of the production fermenter. I told you that if the if the production fermenter size is 100 cubic metre then 5 per cent is about 5 cubic metre and 10 per cent is about 10 cubic metre.

The volume of inoculum for this fermentation process is about 5 to 10 cubic metres so usually what we do we take 90 cubic metre of the media and 5 to 10 cubic metre of the inoculum we inoculate to make the volume of 100 cubic metre.

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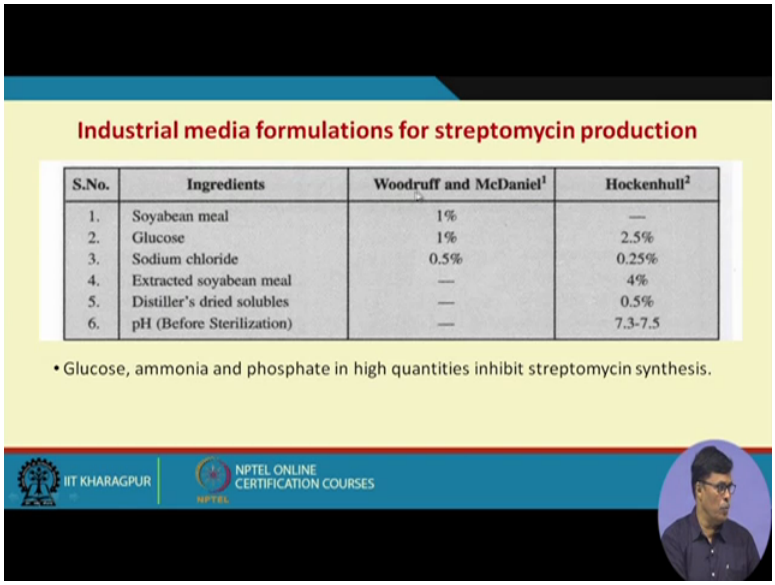

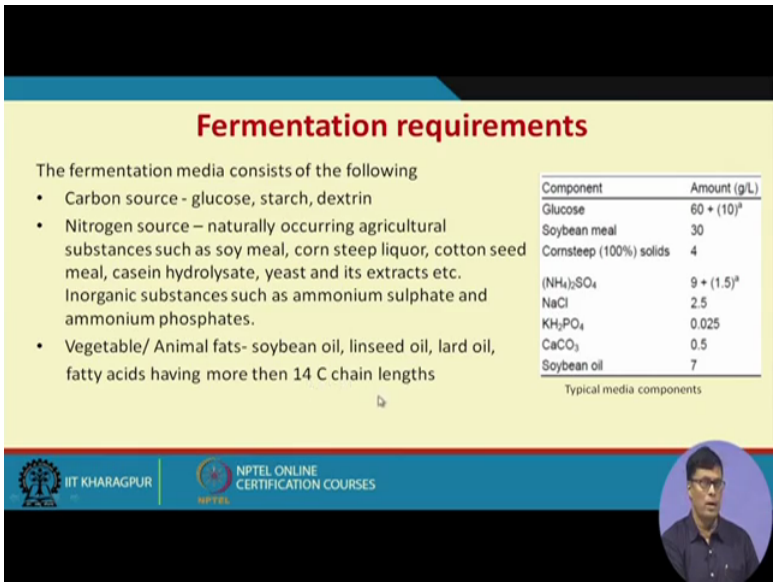

Now this is the industrial media formulation for streptomycin production we have two media one is Woodruff and McDaniel and another is the Hockenhull this media, then this media

contains the soya bean meal but it does not contain the other media does not contain soya bean meal, but it has the extracted soya bean meal.

So this is this is total soya bean meal that is here use here and Distiller's dried soluble that is also and pH we maintain here 7.3 to 7.5. Glucose ammonia and phosphate high quantity inhibit the streptomycin production if you look at this fermentation requirement media consists of carbon source like glucose, starch and dextrin. Nitrogen source natural occurring agricultural substances such as soya bean, corn steep liquor, cotton seed meal, casein hydrolysate, I told you casein hydrolysate means casein is a kind of protein. When casein we hydrolyse with the help of some kind of protease enzyme we decrease it to the different that size of protein or it may produce some kind of free amino acid also.

So this is this is that is that is kind of soluble material the because when big protein molecule degrade to the smaller protein molecule it is becoming soluble and that is why we call it casein hydrolysate. Yeast and its extract et cetera, inorganic substances such as the ammonium sulphate and ammonium phosphate. The vegetable and animal fats like soya bean oil then linseed oil and lard oil that is used, the fatty acid having more than 14 carbon chain length. Because I told you one common thing that we have in the in the microbial fermentation process is the foam formation. And particularly when we have aerobic fermentation we have a foam formation and during foam formation I told you that this is keep on rising and if it enter into the mechanical cell your system will be contaminated.

And then the whole fermentation process will suffer, you have to discard the all fermentation broth. So this is a important thing that everybody has to consider that how to subside this foam and I told you before also the different fermentation process we use the different antifoam oil, because we shall have to we choose the antifoam oil on the fact that the kind of antifoam oil that we use that should not affect the fermentation process. In case of streptomycin fermentation process they found the soya bean oil, linseed oil and lard oil which has for more than 14 carbon chain they are suitable for as a that and that as the antifoam oil.

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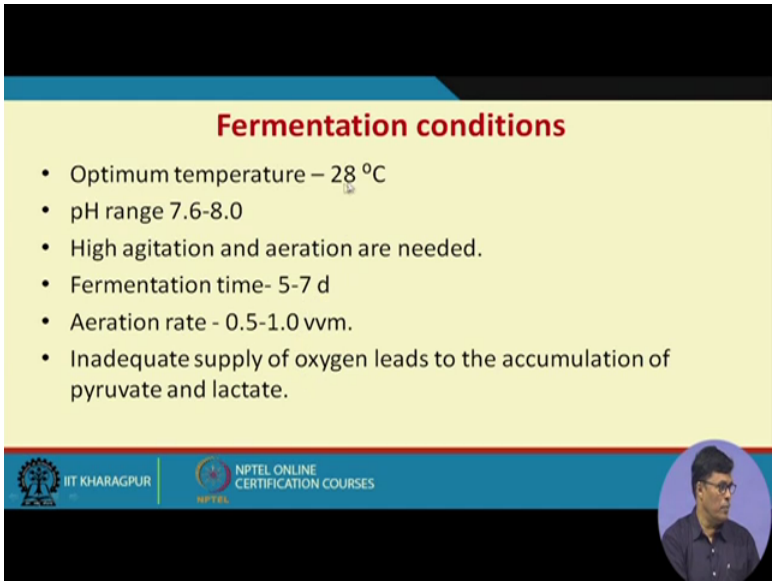

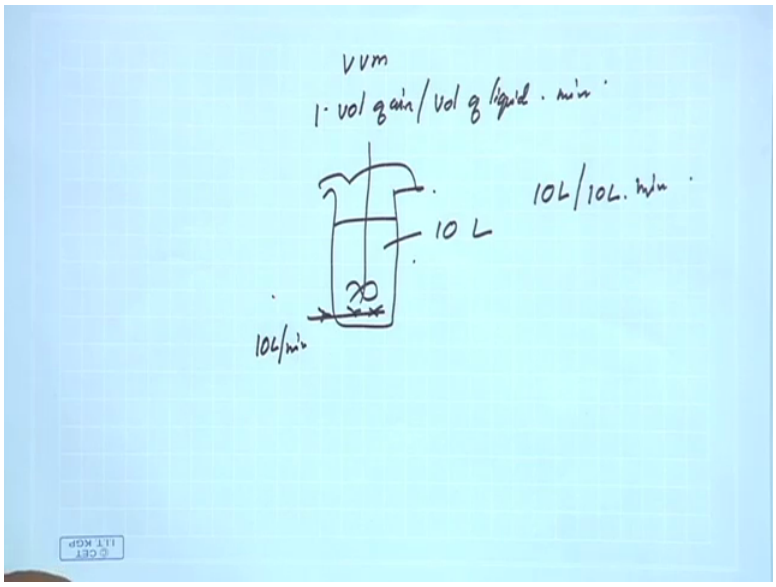

Now fermentation condition are that optimum temperature 28 degree centigrade, pH range is 7.6 to 8. High agitation and aeration are required, the fermentation time is 5 to 7 the days. Aeration is point 5 to 1 vvm, this I want to point out that vvm means the I hope you might be knowing vvm means volume of air per volume of liquid per minute so it is like this. Suppose we have this is the fermenter and the working volume let us assume this we are doing the aeration, air in so let us assume this working volume is 10 litres and if you say this is 1 vvm that means 1 litre the 1 vvm means 10 litres if 1 vvm then 10 litres per 10 litre volume of the medium per minute. This is like this this is how the aeration is considered.

So we shall have to the flow rate of this here the flow rate will be 10 litre per minutes so since it is capacity 10 litres so vvm will be 1 vvm is like this. Inadequate supply of oxygen

leads to accumulation of pyruvate and lactate that is also very important factor. The phase involved in the streptomycin fermentation several phases are there, that phase 1 it last for 24 hours, pH increases to 7 point 5, this highly active the proteolytic property of the organisms set free the ammonia and that into the medium of the soy bean meal.

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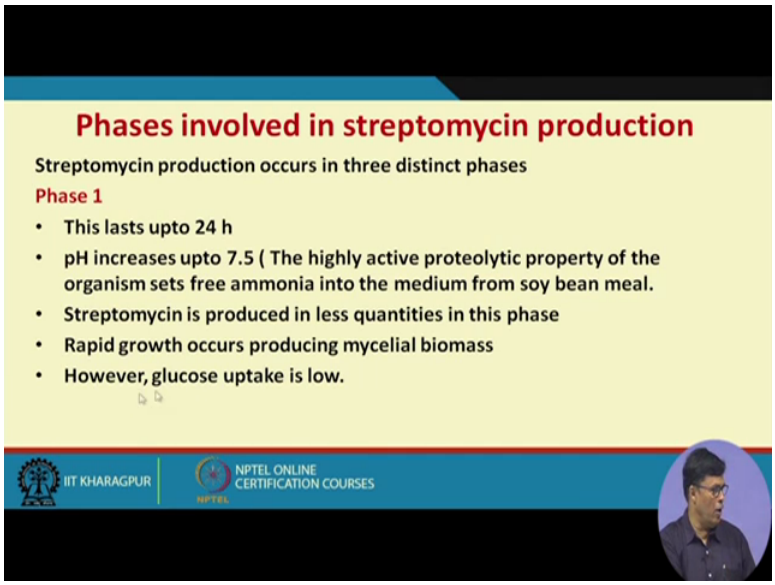

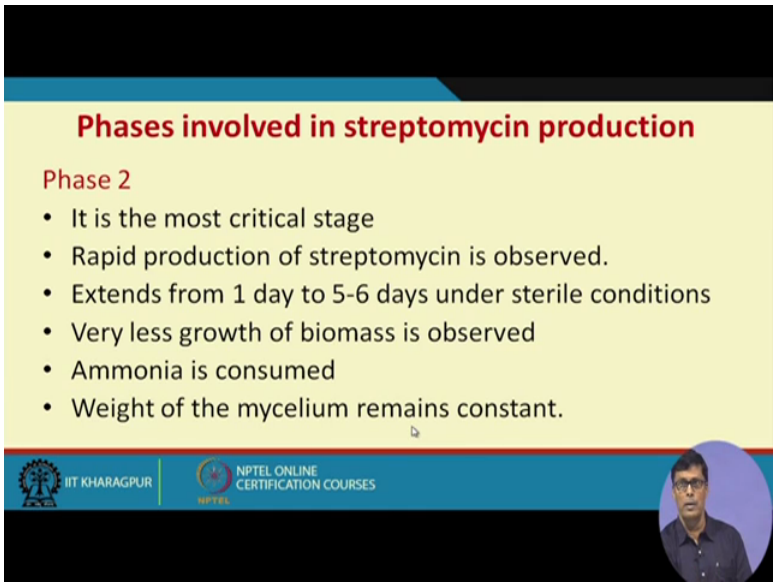

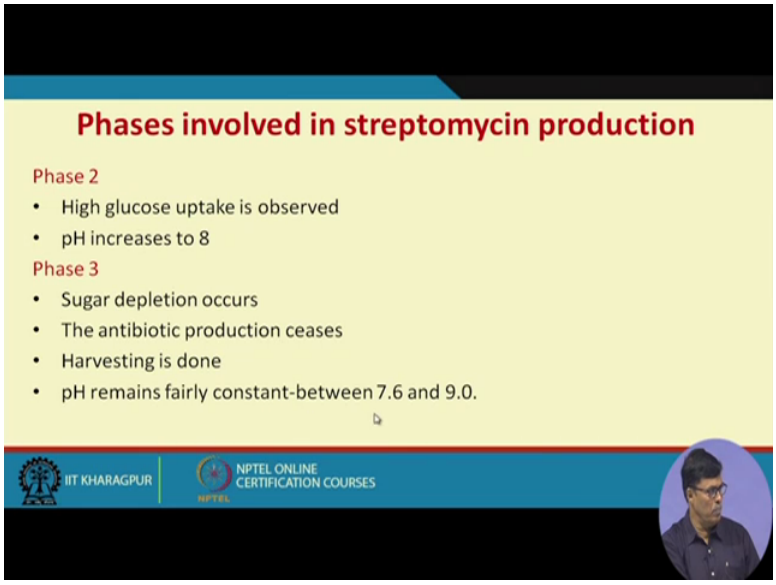

And this this due to the formation of the ammonia the pH rises. Streptomycin is produced less quantity at this phase and rapid growth occurs to produce mycelium biomass, and we have already shown though it is the bacterial cells but it the growth characteristics little bit similar with the fungal cells. However, glucose uptake is low now in case of phase 2 it is most critical rapid production of streptomycin is observed, extends for 1 day to 5 to 6 days under sterile conditions and then very less growth of biomass is observed. Ammonia is consumed and weight of the mycelia remain constant, because this is also a secondary metabolites production because this also takes place at the stationary phase of the fermentation.

Now phase involved and that high this phase the involved the high glucose uptake phase 1, I have shown the glucose uptake is placed, but here is very high, pH increases to 8. And phase 3 the sugar depletion occurs and then antibiotics production ceases. The harvesting is done and pH remain fairly constant between 7.6 and and 9.

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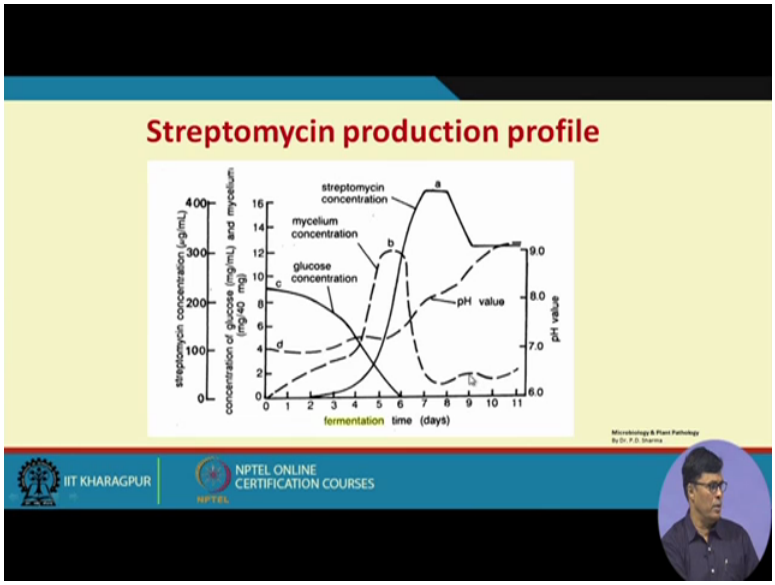

Now if you look at the different profiles of the streptomycin production it looks like this now here it is very very important thing is the glucose if you look at this is the carbon source it keeps on decreasing like this and this is in fermentation time in days. So after 6 days of fermentation most of the glucose will be exhausted. Now if you look at the mycelium or cell mass production it keep on increasing, and then it is it is biphasic type, it increases like this then it increases like this then it decreases like this.

So it is like this then pH if you look at ph is keep on sterile increases the initially it will be at a very slow rate but finally it increases quite high. And here is the streptomycin production is when this organism almost come here it reproduces like this streptomycin production takes place and slowly slowly it increases and then attains the plateau then again decreases like this.

So maximum you can see the maximum streptomycin production that take place on on 7 days of fermentation on 7 days of fermentation, we get the maximum streptomycin production. Now streptomycin recovery also very important aspects because how the streptomycin recovery takes place.

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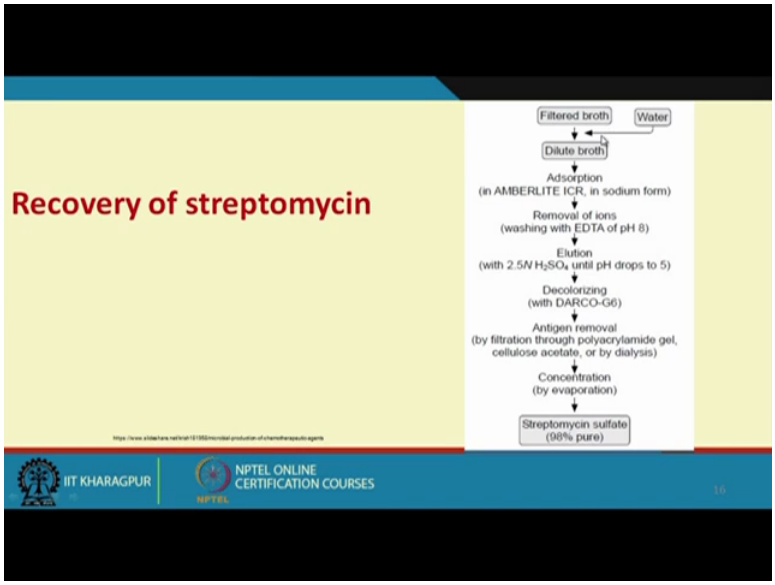

Now this is usually takes place at the adsorption phenomenon now how it is done we have the filtered broth then we separate the cell may be either centrifugation mostly we use by the cell separate by centrifugation process then we have the dilute broth. We we pass through this AMBERLITE this ICR sodium form iron with the cation exchange column we use to pass that, then your streptomycin get the adsorb from the surface, then we remove the ions by washing with EDTA at pH 8.

Then finally we do the illusion by 2 point 5 normal H2SO4 until the pH drop to 5 and then we get the liquid that liquid we decolourise by using with the DARCO G6 column then then antigen removal by filtration with the polyacrylamide gel cellulose acetate or by dialysis. Cellulose acetate you know that this is a kind of membrane that we have and then finally we do the concentration by evaporation technique and after evaporation we get this powder white powder in the form of this streptomycin sulphate. So we get the streptomycin in the form of streptomycin sulphate.

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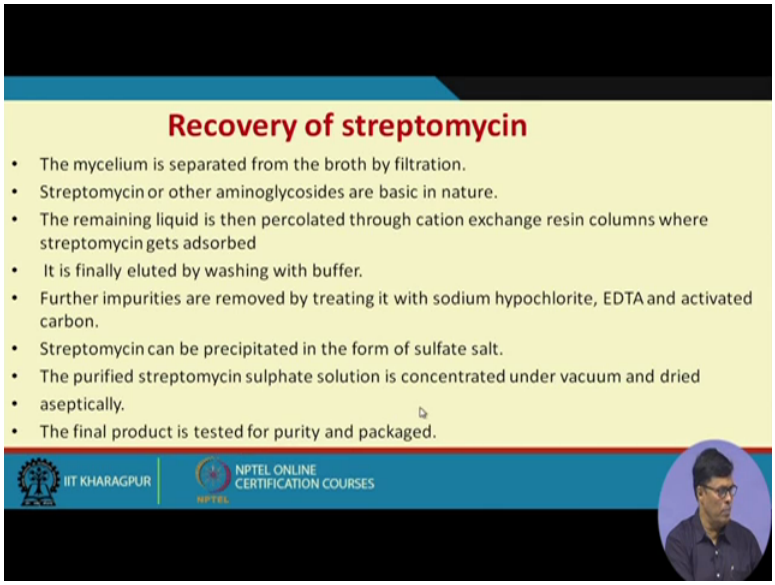

The recovery of a streptomycin is that mycelium is separated from the broth by filtration then streptomycin and other aminoglycosides are the basic in nature. The remaining liquid is then percolated through the cation exchange resin column, where the streptomycin gets absorbed. Then it is finally eluted by washing with buffer and further impurities are removed by treating with sodium hypochlorite or EDTA or activated carbon, because activated carbon I I told you before also largely used by chemical and biochemical industry as a decolourising agent.

And then streptomycin can be precipitated in the form of sulphate when it combines with the H2SO4 it forms the calcium streptomycin sulphate with soluble. And the purified streptomycin sulphate solution is concentrated on the vacuum and dried and and dried it aseptically and finally the product is tested and purity and packaged. So this is this is this is how the streptomycin production takes place because and let me conclude here that streptomycin is kind of antibiotic that is produced through the bacterium fermentation process.

Most of the antibiotics is produced through the fungal fermentation process couple of antibiotics can only produce through the bacterial fermentation, streptomycin griseus whose nature is little bit similar as compared to the fungal cell because it like you know fungal cell it has the sporulation characteristics. And also this streptomycin griseus it has also sporulation characteristics. And when it grows in the liquid it gives the that that that mycelium production that same as in case of fungal cells.

So and this streptomycin also consider as a secondary metabolites that produce and it takes quite long time like you know similar to penicillin fermentation process, penicillin and streptomycin the cephalosporin it 6 to 7 days fermentation process is taking place. After the fermentation is over we separate the cells by through the filtration process and since if the size of the cell is big we can use a rotary vacuum filter for separating the cells and size is small then we use the centrifugation technique and then we pass it through through this absorption column.

Adsorption column the adsorb the streptomycin and then after adsorption we elute with it is the absorbed in the cation exchange membrane and then we elute by using the 2.5 normal H2SO4, so the streptomycin sulphate will goes out. Then we do little bit of purification we remove the impurities that present in this particular elute that we colour we remove. And then finally we dry it by using the vacuum dryer, as we know that that at low vacuum the boiling point of the that water decreases.

So you can you can if you if you do the vacuum, vacuum drying technique then you can preserve the quality of the medium and this whole operation usually done under aseptic condition because it is used for the therapeutic purpose. So this is all about the antibiotics fermentation process I want to cover and try in the next class, next lecture I try to discuss one very interesting fermentation process that is used by the industry that is the Baker's yeast fermentation process. Thank you very much.