Industrial Biotechnology.
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Lecture-41.
Cephalosporin Production.

Welcome back to my course Industrial Biotechnology now today I am going to discuss another antibiotic fermentation process cephalosporin. Now in the last lecture I covered the penicillin fermentation process and penicillin is consider as a beta lactam antibiotic and it has lot of applications particularly I told you after the Second World War that soldiers they wounded and for their for their recovery, the health recovery badly required penicillin which was application was more.

Now penicillium chrysogenum that is the fungal strength that is used for the penicillin production and then we discussed that what are the different penicillin that is largely available. I told you that penicillin G and penicillin B was mostly used and penicillin G is comparatively very much unstable in the acidic pH so it is not recommended to take in the form of capsule because when you take the capsule then it goes to the stomach and pH has the acidic pH of 2.

So naturally the activity of the penicillin will be lost so that is why penicillin G usually recommended to use in the form of injection fluid and penicillin where otherwise that penicillin B that is usually recommend in the form of capsule, now penicillin G can be used for the production of semi different semi synthetic antibiotics like ampicillin and others. So now I discuss that the fermentation process in details then also I discuss the downstream processing how penicillin recovered.

Penicillin is usually marketed either in the form of potassium penicillin or sodium penicillin, that today we want to discuss another beetle lactam antibiotics that is largely used that is the cephalosporin. As compared to penicillin penicillin is a narrow spectrum antibiotic because it is only active against the gram positive bacteria but if you look at cephalosporin C that is active not only in the gram positive against the gram positive bacteria but also gram negative bacteria.

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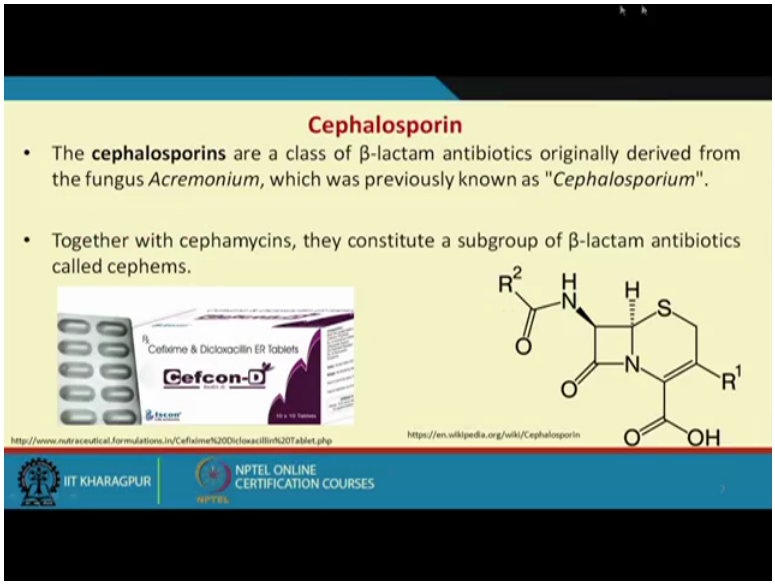

But mode of action of this this cephalosporin and the penicillin is more less same because both affix the cell bond formation of bacteria. So let me go in the details, the cephalosporin is a class of beta lactam antibiotics original derived from fungus called acremonium which was previously known as cephalosporin, together the cephamycins they constitute a subgroup beta lactam antibiotics called cephems. So this is the beta lactam (())(03:47) I I mentioned in the last class and in case of penicillin we can remember this R group is one but here we have two R groups here and here.

And here is a a kind of benzene type of ring that we have the close ring that we have that we do not have that kind of ring in case of penicillin but it is R1 and R2. So different type of cephalosporin that is available in the market. They have first generation second generation third generation cephalosporin just by changing the R1 and R2 groups.

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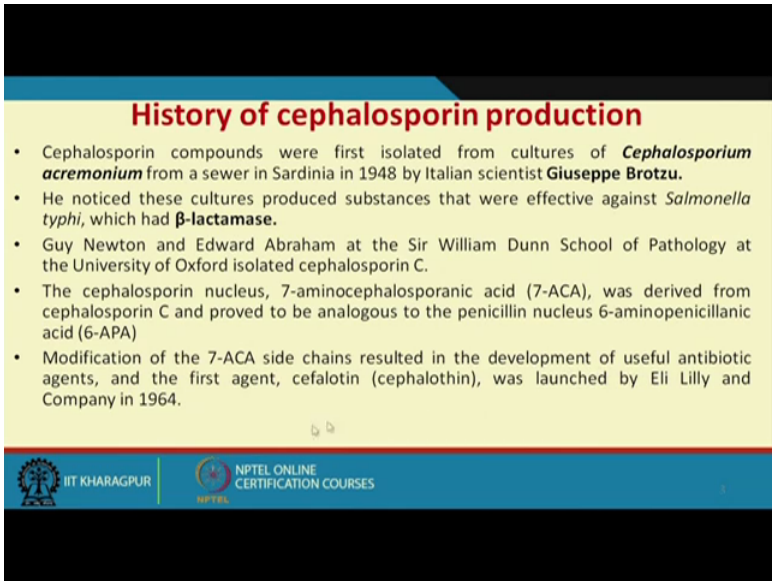

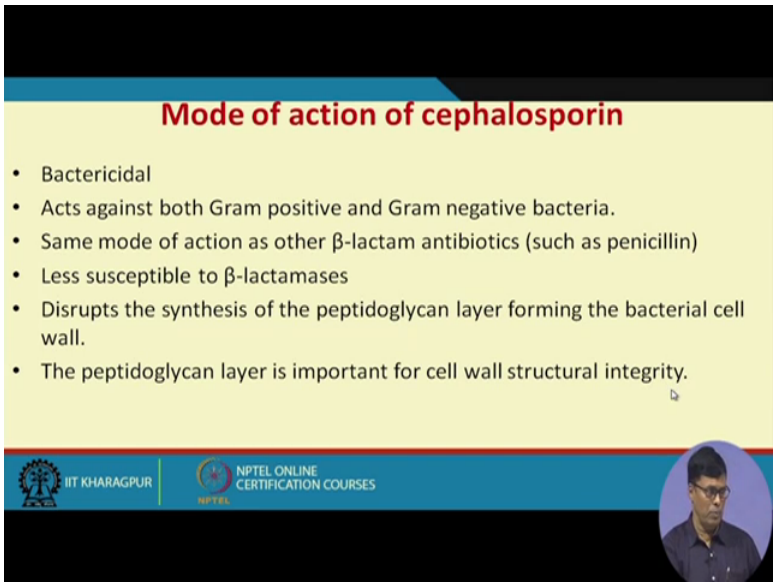

Now if you look at cephalosporin that production cephalosporin compounds was first isolated from the culture cephalosporin acremonium for a sewer of Sardinia in 1948 by Italian scientist Giuseppe that that he identified this organism he noticed that this culture produce substances that were effective against the salmonelia typhi, which has beta lactamase. So salmonelia typhi we know it is kind of pathogen so this is active against this particular bacteria.

The Guy this Newton and Edward Abraham at the Sir William Dunn school of pathology at university of Oxford isolated cephalosporin C. Now cephalosporin nucleus we have 7 aminocephalosporanic acid what you call 7ACA was derived from the cephalosporin c and

proved to be analogous to the penicillin nucleus of 6 aminopenicillinic acid so it has quite risen balance with this 6 aminopenicillanic acid.

Modification of 7ACA side chain resulted in the development of useful antibiotics agent and the first agent is cefalotin what you call cephalothin was launched by Eli Lily and Company in the year 1964. Now let us see mode of action of the cephalosporin, the cephalosporin is it is it is considered as a bactericidal affect acts again gram positive and gram negative bacteria i mentioned before.

Same mode of action as other beta lactam antibiotics such as penicillin I told you that penicillin actually that that affects the cell wall formation of the bacteria. Now less susceptible to beta lactamases enzyme and distract the synthesis of peptidoglycan layer forming the bacterial cell wall. The peptidoglycan layer is important for the cell wall structure integrity because when I discussed about the penicillin then we I discussed about that. The organism that is used for the cephalosporin production that is cephalosporium acremonium later renamed as acremonium chrysogenum is used.

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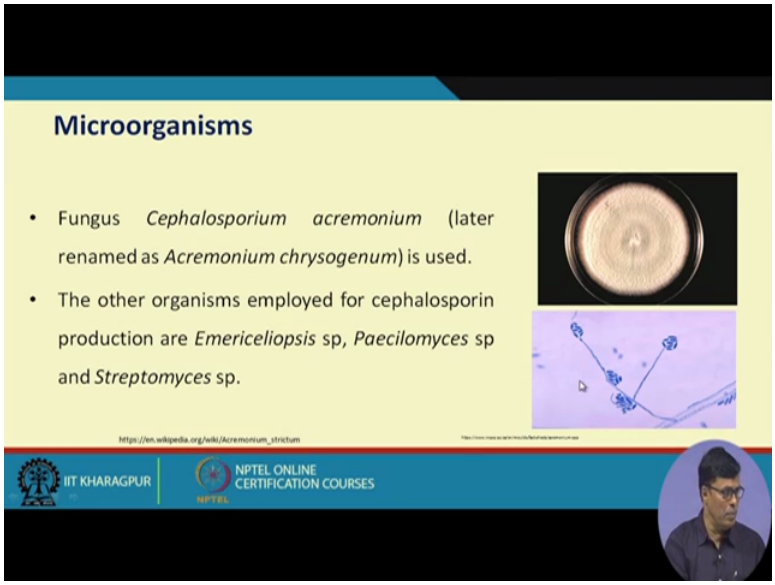

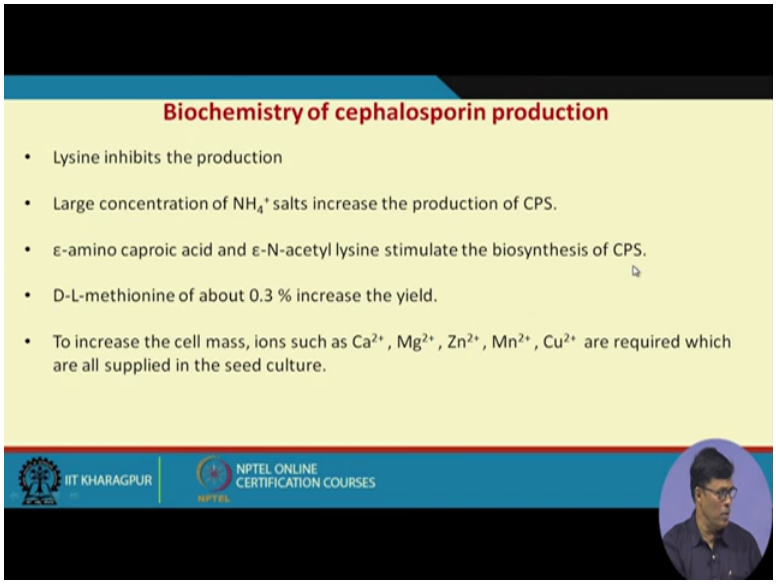

And other organisms employed for the cephalosporin production is Emericeliopsis and paecilomyces and Streptomyces species so difference species and if you at the morphology it is something different you see there is a stem is there and and this some kind of spores you can see at the end kind of formation is there the stems. Now if you look at the biochemical synthetic pathway for cephalosporin it is usually produced for alpha L alpha amino adipoic acid LIC L Valine when they combine together they produce this compound what you call ACV.

And then from this we produce isopenicillin N and this isopenicillin N forms the penicillin N and then it forms DOOC and DAC ultimately it produce the cephalosporin, C this is the compound we have as per cephalosporin C is concerned. Now the biochemistry that in this this cephalosporin production the Lysine inhibits the production of the cephalosporin large concentration of ammonium salt increase the production of cephalosporin and eta that amino caproic acid eta N-acetyl lysine stimulate the biosynthesis of CPS that is the cephalosporin S and D-L methionine this is the important sulphur amino acid that affects the cephalosporin fermentation to a great extent.

The one important component required in the medium of this organism that is for the production of cephalosporin is methionine and which is which required about point 3 per cent to increase the yield, to increase the cell mass ions such as the calcium, magnesium, zinc, manganese, copper are required which all are supplied in the seed culture.

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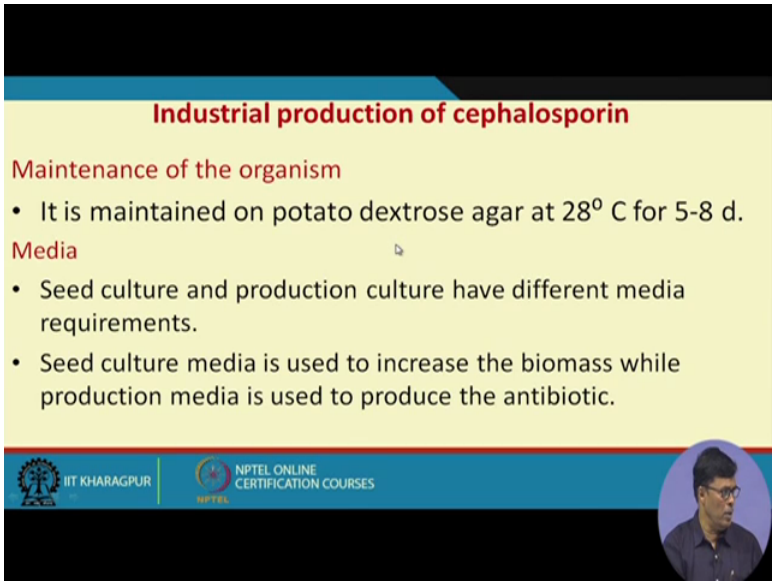

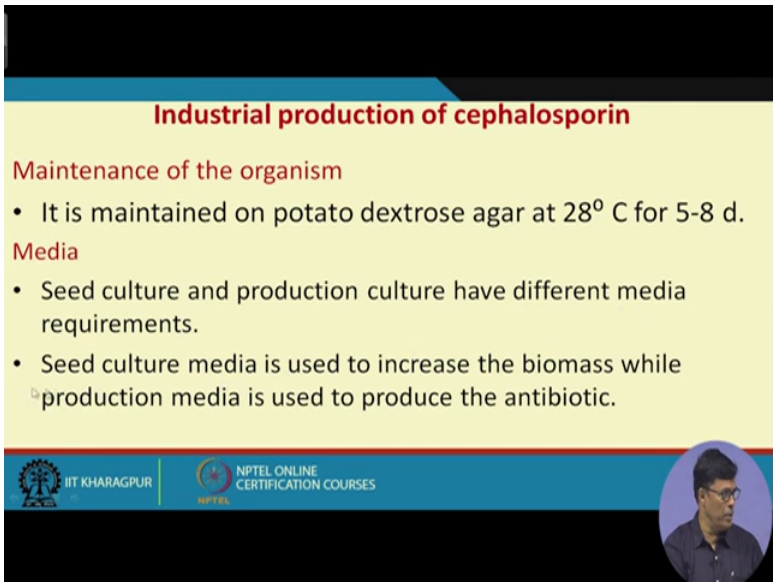

Now industrial production as I told you that for industrial production we require the industrial strain, and industrial strain basically it is little bit different as compared to wild strain, wild strain they do not cannot withstand the adverse condition but the industrial strain they can withstand the adverse conditions like temperature sudden change of temperature, sudden change of pH. Not only that most important thing is that the productivity is very high and also it does not change with the generation and stability of that genetic stability of the organism should be very high.

Now maintenance it is usually mentioned in the potato dextrose agar at 28 degree centigrade for 5 to 8 days. This is we can maintain there then seed culture and production culture have different media requirement I told you that for the growth of the most of the cases we seen

the growth of the cells and the production we required the different because purpose of the growth media and purpose of the production media is totally different.

Purpose of the growth media is to we are interested to produce more cell and purpose of the growth media we are interested to produce more product. So that is now in case of Baker's yeast fermentation process I shall discuss after some maybe after one lecture there I shall this is when field is the product and then the situation will be totally different. Then then the composition of the inoculum vessel and composition of production fermenter they will they will be same.

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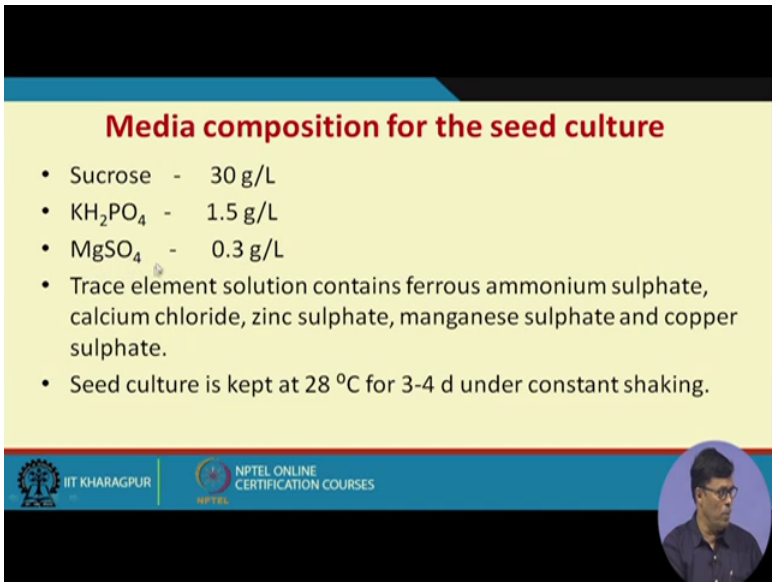

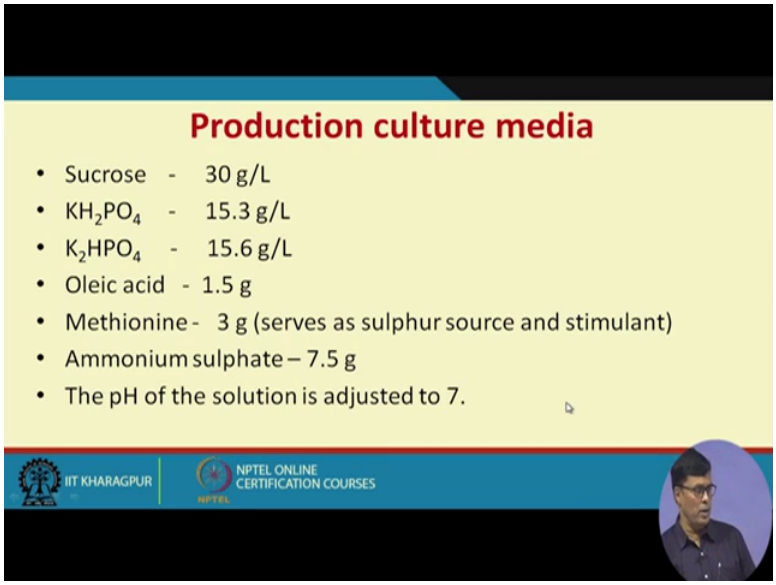

And as far as media is concerned the seed culture and production culture has different media requirement. Seed culture media is used to increase the biomass while the production media

used to you know to produce the antibiotics. The media composition for this seed culture contains the sucrose 30 grams per litre that potassium dihydrogen phosphate 1.5 gram per litre, magnesium sulphate point 3 gram per litre.

Trace element solution containing the ferrous ammonium sulphate, calcium, chloride zinc sulphate, magnesium sulphate and copper sulphate is required. The seed culture is usually kept at 28 degree centigrade for 3 to 4 days under constant shaking, so under stirred conditions. Now in the production media we require little bit different we can see it but one mandatory thing is that it should have oleic acid and the methionine because I told you methionine also it require for the growth of the organism required ammonium sulphate and pH of the solution adjusted to 7.

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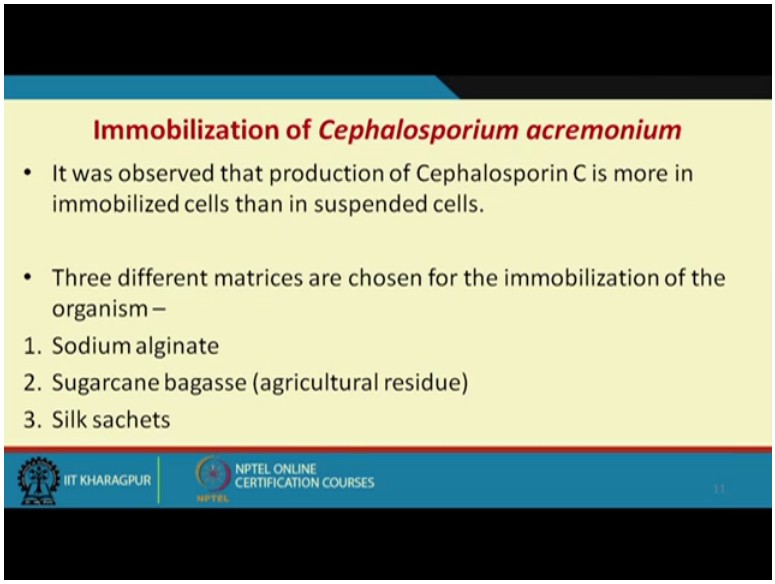

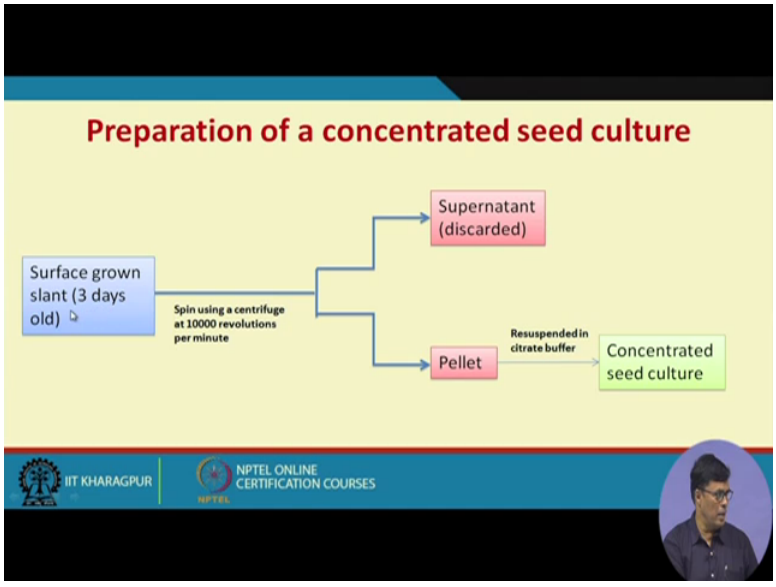

Now this can be produced in two ways both it can be produced by using suspended culture also it can be produced by using immobilise culture now I shall I shall hear one interesting thing is that through immobilization we can produce cephalosporin let me explain how it is done. It was observed that the production of cephalosporin is more in the immobilized cell than in the suspended cell.

Three different matrices are chosen for the immobilization of the organism where during I think we when we discussed the immobilization technique we already we discussed what should be the characteristics of the solid matrix and how we do the immobilization. So here will find how it has it is used in the industrial level. Now the one is sodium alginate largely used this is used entrapment technique they use for the immobilization of the cells, then sugarcane bagasse this is used the immobilization cell through the process of this adsorption phenomenon then silk sachets that is also used for the immobilization of the of the organisms.

And so this is this is the preparation of the seed culture where surface grown slant three days old we take it out and then we we we centrifuge that supernatant we we discard the pellet we take it out and re-suspended in the citrate buffer we this is the citrate the seed culture which is used for the inoculum, inoculation purpose.

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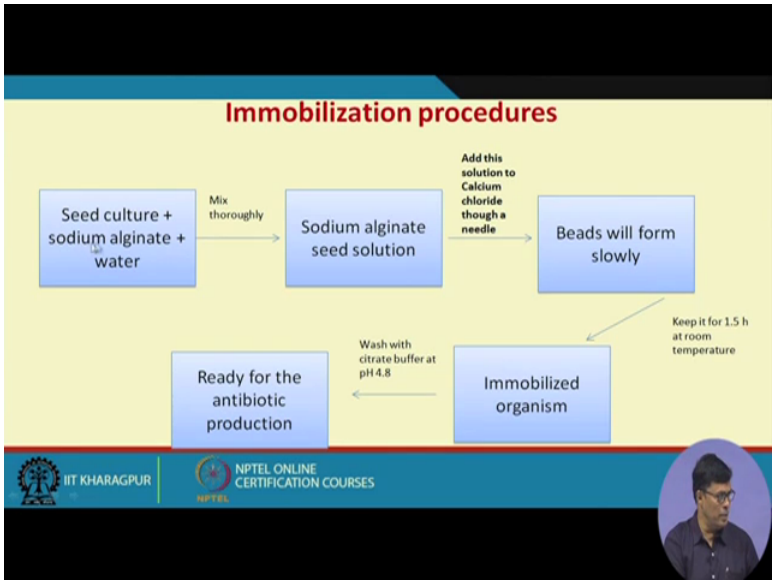

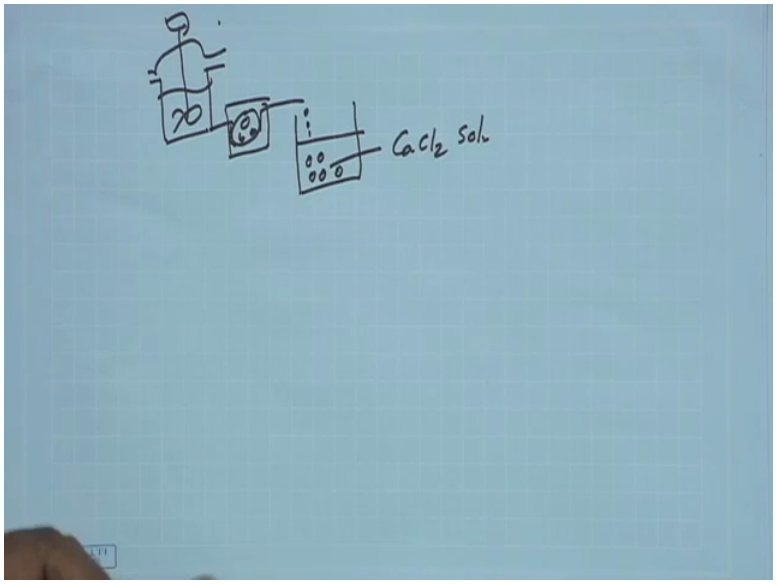

Now this seed culture and sodium alginate and water we make a mixture and then this this sodium alginate mixed thoroughly and sodium alginate seeds solution we we put it drop by drop I can this this can be done automatically with the help of pump because suppose we had peristaltic pump. We have like this the suppose we have this reactor we make a solution then it pass through this this is the pump that we have, it comes and it falls drop by drop. Now here we have calcium chloride solution. So when it falls drop by drop it forms the pellets like this.

The beads formation is there kind of this kind of bead formation is there bead will form slowly and keep it for one and half hour at the room temperature immobilize organism wash with citric buffer at pH 4.8 ready for antibiotics production. So this is how immobilization is done and this technique is called entrapment technique, inside the solid matrix the cells are entrapped.

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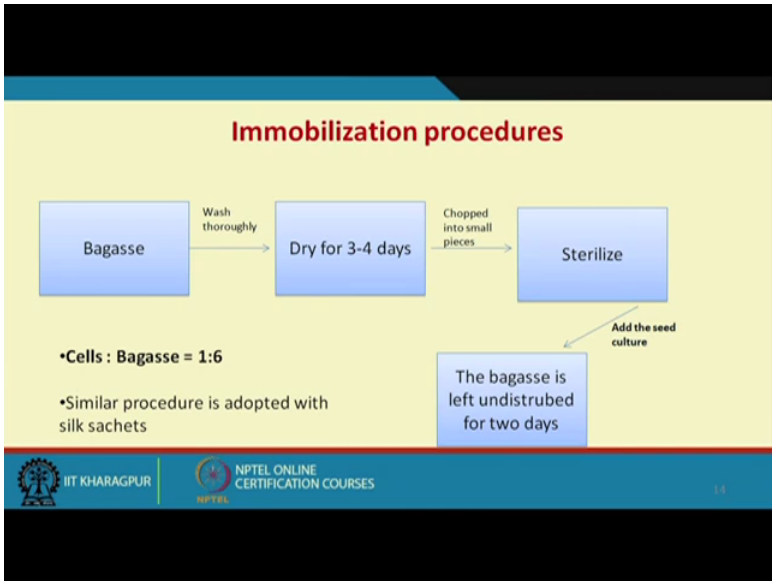

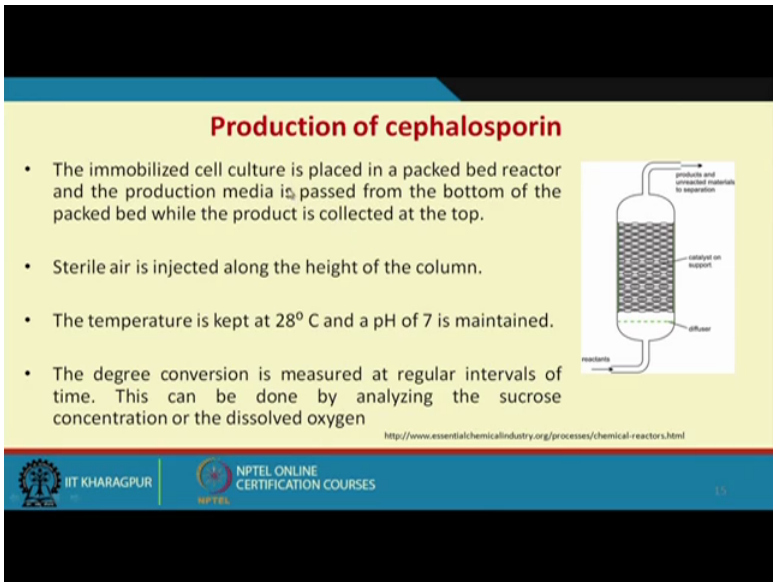

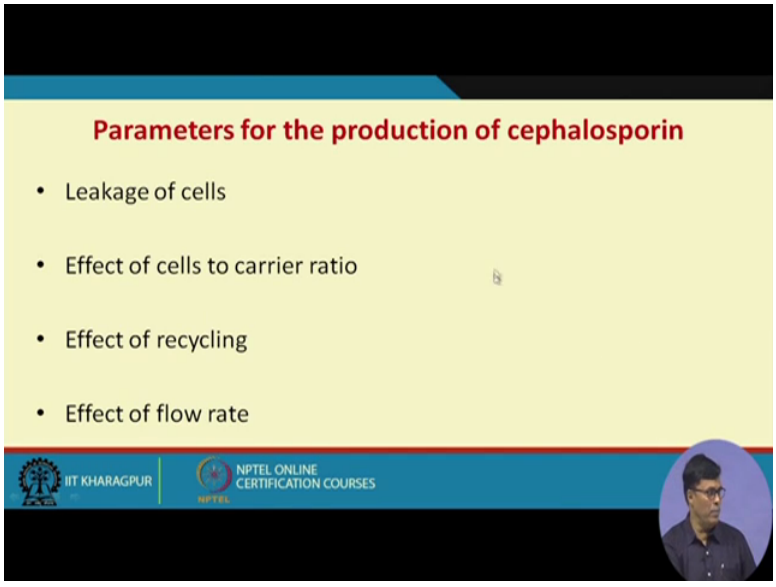

Now in case of bagasse that how we do it we wash it thoroughly then dry it for three to four days then chop to the small pieces because more surface area more will be adsorption because the adsorption that you know that immobilization of the whole cell depends on the surface area more surface area more will be the adsorption of the cells and then we sterilize that and then this bagasse left undistributed for undisturbed for two days.

The similar procedure as adopted and this we pack in a column we pass the solid matrix and immobilize that on the surface. The immobilized culture is placed in a packed bed reactor this is the solid material where the immobilize cells are immobilized and and this production media pass from the bottom we we pass the media from the bottom we take out the border product from the top and the sterile air is injected along with the height of the column, because the air is required the reason is that this is the aerobic fermentation process the temperature kept is 28 degree centigrade and pH is 7 is maintained.

Degree of conversion is measured at regular interval of time this can be done by analysing the sucrose concentration and the dissolved oxygen because we can easily find out how much this product formation we time to time we draw the sample and find it out. The parameters that affect the cephalosporin production process with the leakage of cells, the effect of cells to carrier ratio, effect of recycling, effect of flow rate.

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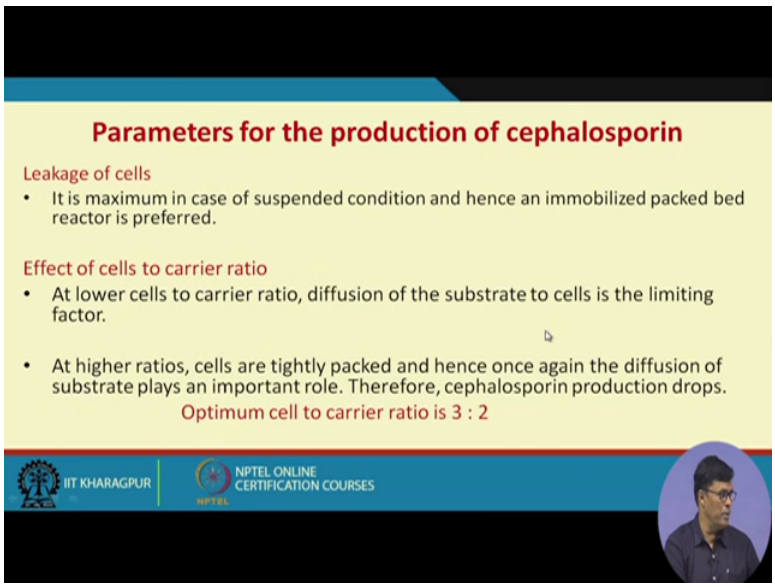

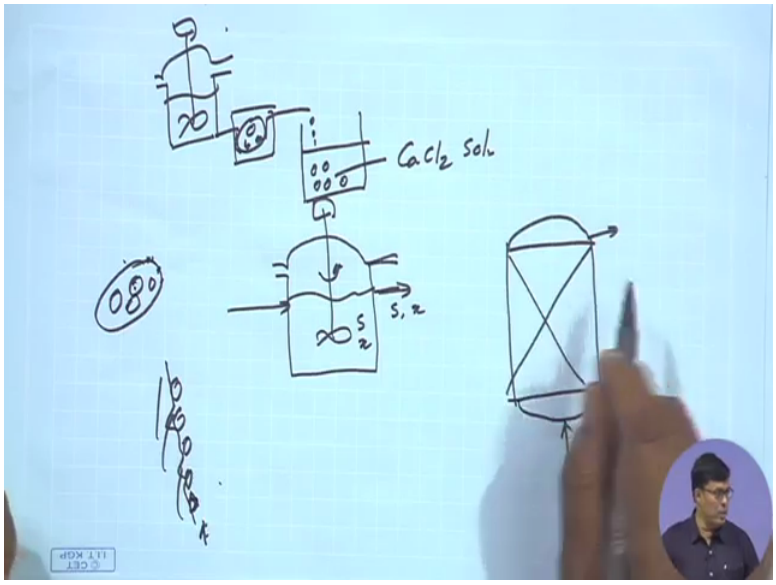

The leakage of cells that is very important the maximum it is maximum in case of suspended condition and hence the immobilized packed bed reactor is preferred. So so I can I can I can say you here I can explain little bit then I told you in case of CST reactor when liquid is going out so here you have substrate you have cell mass so both the cell mass and substrate when it liquid is going out it carries this cell mass with that. So you will be continuously we are losing the cell from the reactor but when you when you have a column like this and you immobilize the cell here and take the product out.

So you are not allowing because you know that suppose I told you that you can put the cell inside the solid matrix like this or you can you have the solid matrix like this and on the surface your cells may adhere that also. Since it is adhere so it is a supporting material now this is this is the adsorption phenomenon is a surface phenomenon because if your shear force is very high then it will take the material out but in case of this when the cells are entrapped cells cannot go out until unless the porosity of the of this this solid matrix is very high otherwise it will be went inside the solid matrix.

So this is this is thing that is called the leakage of the cell, the leakage of the cell in case of suspended conditions will be more as compared to that of immobilize conditions. Effect of cells to carrier ratio is important because how much cells are there per gram of carrier carrier means solid matrix that is the more the cell density we can we can expected the more will be the rate of reaction because cells are the the responsible mostly responsible for carrying out the reaction.

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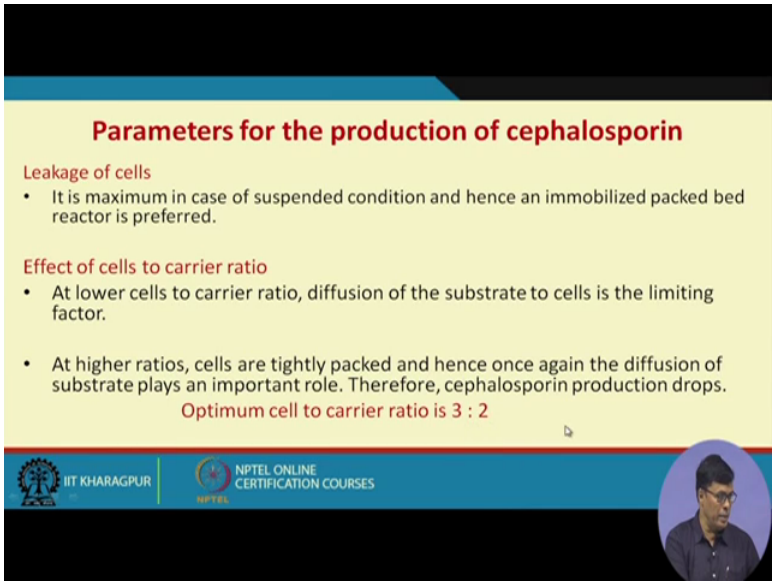

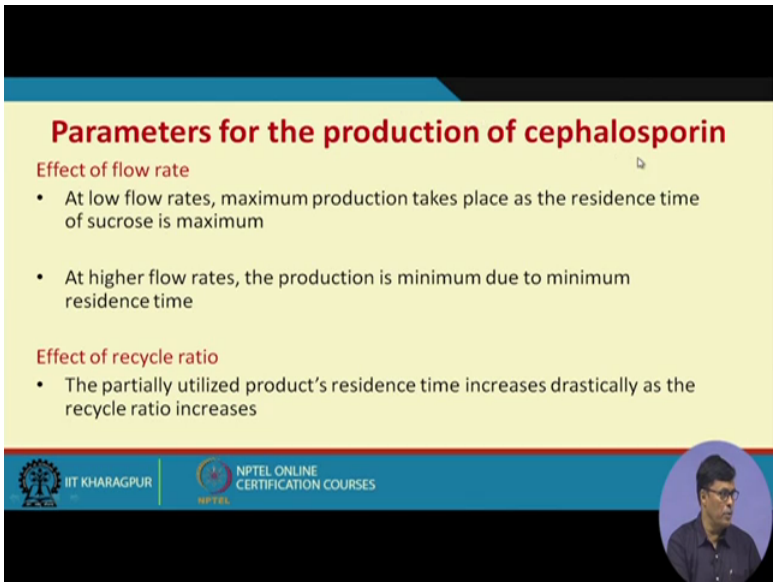

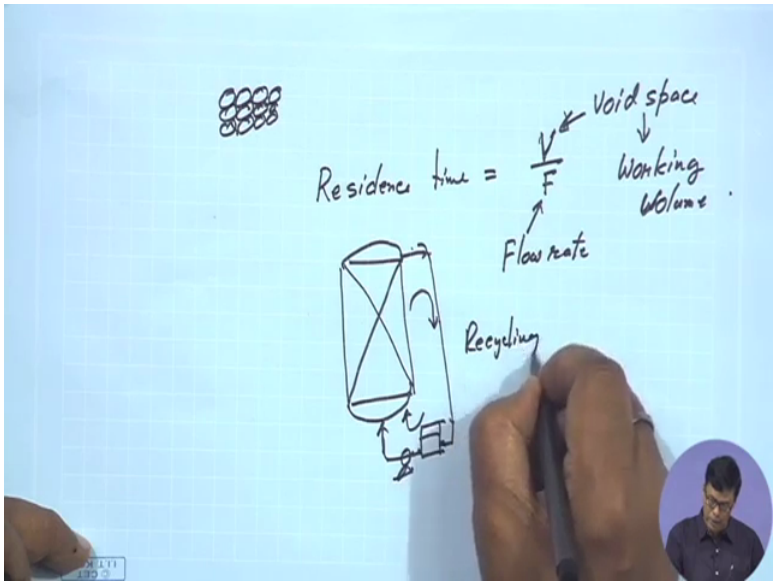

At higher ratios the cells are tightly packed and once again the diffusion of substrate plays an important role. Now that you know that this is this is also I want to I want to tell you that if the cell if the cells it is packed tightly because you are very close like this then in that case the diffusion will be a problem because diffusion against this substrate will give problem. So the so that is why cell by carrier ratio plays a very important role in this in this cephalosporin production. But in case of if we keep on rising the ratio that does not mean your cephalosporin production will increase after sometime we will find the cephalosporin production will drop down.

The optimum cell and carrier issue is found to be 3 is to 2, 3 is to 2 means 3 gram of cells per 2 grams of carrier this is 3 is to 2 that we have. Now parameters for the production of cephalosporin the another effect of flow rate because more flow rate I told you the residence time equal to V by F, F is the flow rate and V is the void space and this void space is nothing but this is working volume of the reactor this is called working volume.

So if we increase the increase the flow rate then what will happen this residence time will keep on decreasing because V is constant for a particular rector if it is constant then your residence time decreases now if your residence time decreases your time you are giving for carrying out the reaction will be reduced, if the time is reduced then your reaction will be reduced, kind of reaction that is taking taking place in your system that is also drastically reduce.

So this is then what we have to do we have to suppose this is a this is a column, suppose you increase the flow rate so if residence time more then what we have to do, you have to you have to take it in the put it in the reactor you have to take it here and again you have to pump it back here. So you know or can I can this is like this you can you can put it in the tank and with the help of pump you can take it to the, so you can you have to recycle again and again, you have to take it back to the what you call recycle, recycling is required.

So if you increase the flow rate then you have to do lot of recycling at low flow rate maximum production takes place as the residence time of sucrose is maximum. But at higher flow rate, the production is minimum due to minimum residence time. Now the effect of recycle ratio, the partially utilize the product the residence time increases drastically as the recycle ratio increases. So in case higher higher flow if you want to increase the residence time just we increase the recycle ratio so residence time increases.

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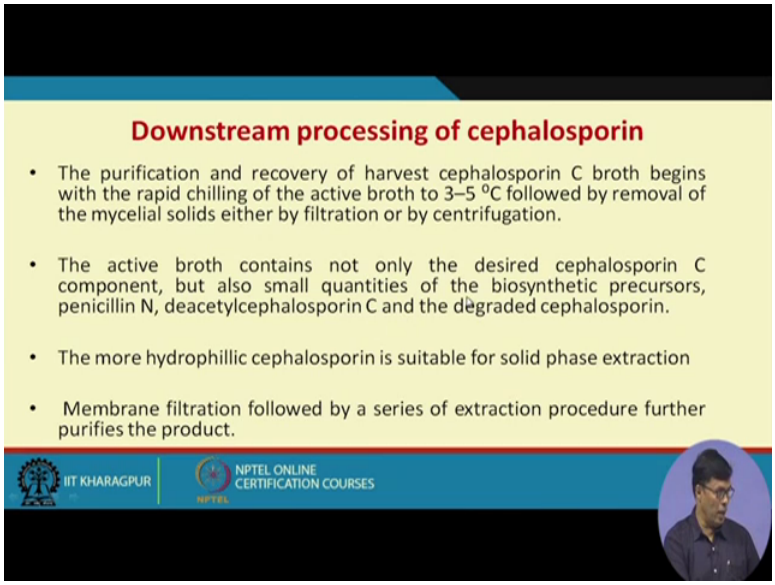

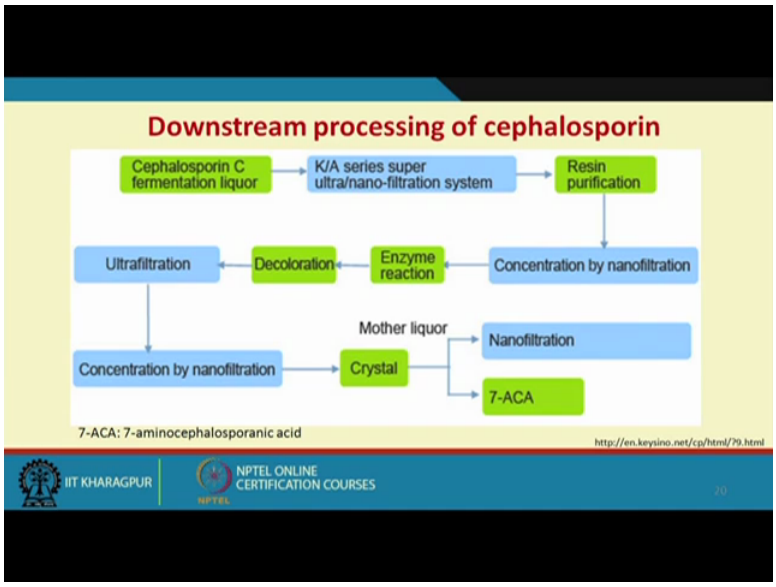

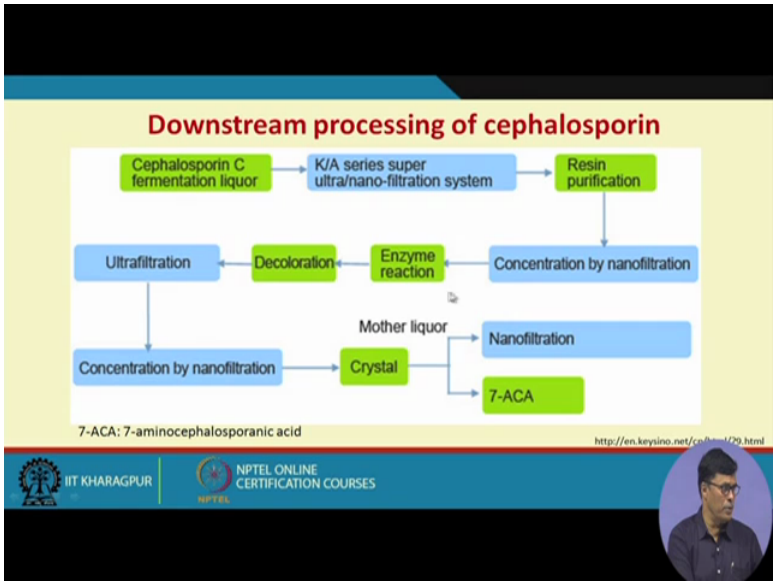

Now let me talk about the downstream processing of cephalosporin the purification and recovery of harvest cephalosporin C broth begins with the rapid chilling of the active broth to 3 to 5 degree centigrade followed by removal of mycelium solid either by filtration or by centrifugation. The active broth contains not only the desired cephalosporin C component but also small quantities of biosynthetic precursors, penicillin N and deacetylcephalosporin C and the degraded cephalosporin. So it contains different type of other material also but it is very present very small amount.

The more hydrophobic the cephalosporin is suitable for the solid phase extraction. Membrane filtration followed by a series of extraction procedure further used for the purification of the product. That we have seen in case of penicillin fermentation process solvent, solvent extraction process, liquid-liquid extraction process were used for the purification of the penicillin.

Now if you look at the downstream processing of the penicillin of the cephalosporin is like this we take the h the cephalosporin fermentation broth here and then we this is I told you I told you that this is fungal fermentation process and size of the fungal cell is quite big, so we use the rotary vacuum filter for the separation of the cell mass from the fermentation broth. Now after here actually we we get the after separation of the cell mass we get the fermentation liquid, then we pass through some ultra nano filtration system then purification with the help of resin purification.

Concentrated by nano filtration enzyme reaction decolouration by activated charcoal type of materials, then ultra filtration concentration by nano filtration then we crystallize that. And we mother liquor we pass it through the nano filters and crystals we have 7 amino cephalosporinic acid. So this is this is how cephalosporin production takes place. So in conclusion I want to say that that like penicillin fermentation process the cephalosporin has tremendous potentiality, only the difference is that penicillin is nano spectrum antibiotics and cephalosporin is a broad spectrum antibiotics, because penicillin is active against the gram positive bacteria but cephalosporin is active against the gram positive as well as gram negative bacteria.

Now as well as well manufacturing process is concerned that little bit different as compared to penicillin. Penicillin mostly fermentation process mostly takes place with the help of suspended culture but here we have shown that cephalosporin we largely produce through the immobilization system. The immobilization can be done by different ways whether

absorption absorption technique as well as the entrapment techniques and then we have to do the purification of the product and cephalosporin C, I forget to mention that as such it is it is not a stable in acetic pH but some cephalosporin we find some kind of first generation, second generation some derivative of cephalosporin when you change the R1 and R2 groups of the that you know cephalosporin molecules, then it is it can withstand the acidic pH also.

That means it can take orally also, when it is resistance to acidic pH then you can take it orally. So this is all about the cephalosporin fermentation process, next I shall discuss about the streptomycin fermentation process. Thank you.