Course on Industrial Biotechnology
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Module 08
Lecture No 40
Penicillin Production (Continued)

Welcome back to my course industrial biotechnology. Now in the last lecture I was discussing about the penicillin fermentation process, I told you that penicillin was discovered by Alexander Fleming in the and then it was the use of the penicillin was actually started after the Second World War, because penicillin is a narrow spectrum antibiotics and it is active against the gram positive bacteria. And penicillin actually that it inhibits the cell wall formation and that is how the cell killed, but it does not affect our intercellular component present in our system.

So now penicillin is first this that organism that microorganism that was discovered which produces penicillin that is Penicillium notatum and then this Penicillium notatum after that switchover to the penicillium chrysogenum for the improvement of the penicillin production. Now for penicillin production it is similar as compared to the citric acid fermentation process here also we have to prepare the culture in the form of in the lab you have to prepare the culture in the form of spores and then spores you have that you put it in the seed tank, after seed tank bring it to the fermentation plant and then you transferred to the inoculum vessel where the vegetative cell will form and this vegetative cell you transfer to the production fermenter.

And then the production fermenter after the usually the fermentation time in the inoculum vessel is varies from 30 to maybe 40 hours and in case of production fermenter it is about 125 to 140 hours or 44 hours. So the it require different carbon source, different nitrogen source, corn steep liquor was found very good nitrogen source as per penicillin production is concerned and as per carbon source is concerned, lactose is find very important carbon source and glucose is used as a source of energy. And but I told you that as it has little difference as compared to other fermentation process the reason is that it require the precursor, as for example for penicillin-G production we required penicillin that phenyl acetic acid, in case of penicillin-V formation we required phenoxy acetic acid.

Now I told you that downstream processing observe the penicillin also quite interesting. We shall have to use the solvent extraction process, solvent-solvent extraction process to purify

the penicillin ultimately you we add some kind of potassium salt in it to precipitate out penicillin potassium or sodium salt in it, to precipitate out penicillin in the form of sodium or potassium salt. Now penicillin is usually marketed in 2 different forms either in the form of capsule or in the form of injection fluid. Let me go ahead with this and let me see that how exactly the penicillin produced by the industry, how it is produced.

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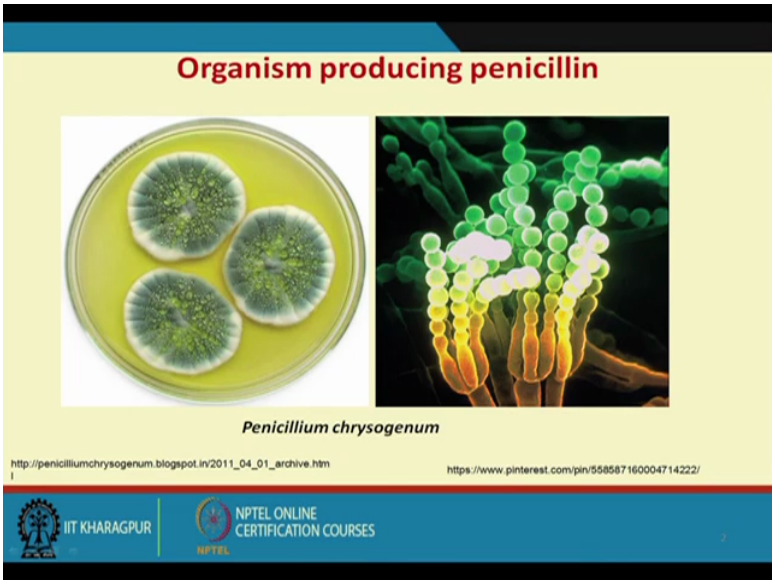

Now first let me I as I told you penicillin it produces beautiful spores, can you see it? This is a very green spores, they are very beautiful looking actually. I told you in case of I have shown you the spores of this Aspergillus Niger that is totally black, but there is a green spore. Now if you see in the microscope, that you will see like this, this is the candy of spores so this is kind of the kind of branches we have we can see that how penicillium crysogenum looks.

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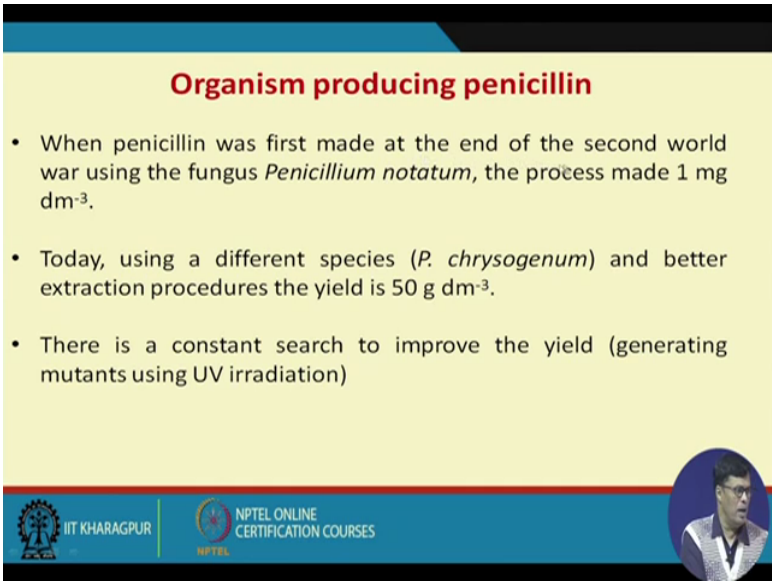

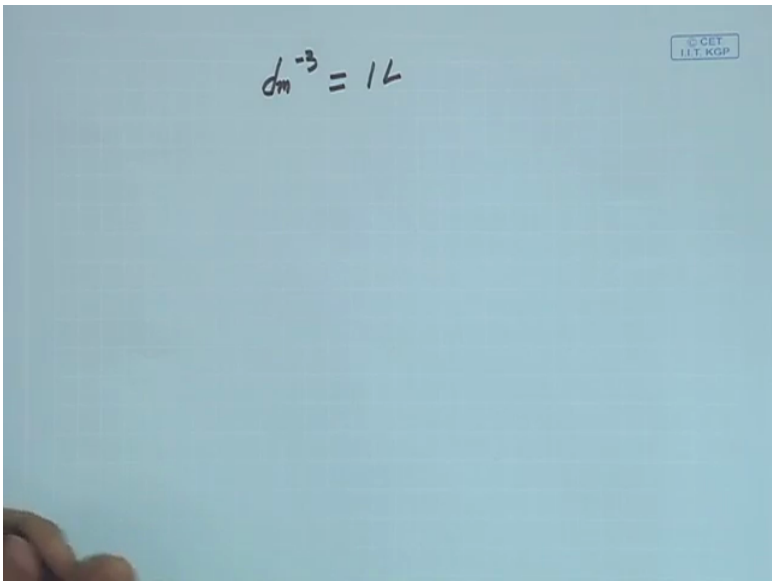

Now, when penicillin first made at the end of Second World War using the penicillin notatum, the process made 1 milligram per decimetre cube. 1 decimetre cube is equal to equivalent to 1 litre. Because that I have to write it here dm decimetre cube is equal to one litre. So 1 gram milligram per litre was the initial concentration of penicillin that was first we found out in the fermentation broth.

Now today you will be surprised to know that by using different species with some genetic modification better extraction procedure it is possible to increase the penicillin concentration as high as 50 grams per litre or decimetre cube. The this is a constant there is a constant search improve the yield generating mutants using the UV radiation irradiation, because not

only UV irradiation, we use different kind of genetic modification we also carry out in the system.

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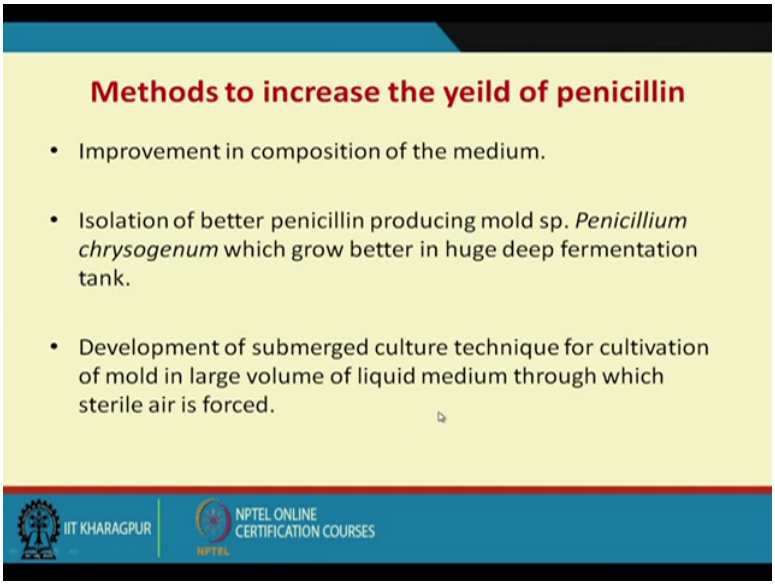

The method increase the penicillin yield, the improvement of the composition of the media, by improved composition media it is possible to increase the penicillin yield. Then isolation of better penicillin producing moulds like Penicillin Crysogenum which grow better in huge deep fermentation tank and development of submerged culture technique for the cultivation of mould in large volume liquid media through which the sterile air is forced. So I initially I because you know this fungi can be can grow both in the surface culture and submerged culture. We find for the penicillin fermentation process submerged culture is best for the penicillin production.

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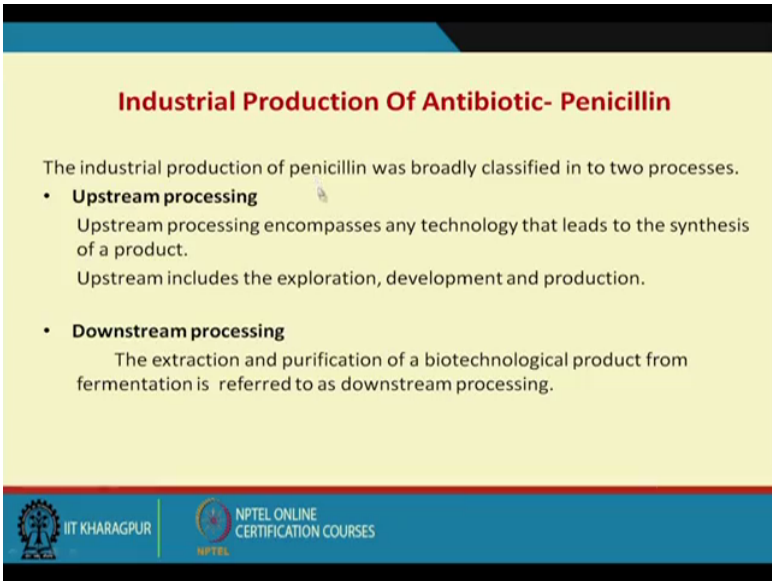

Now industrial production of antibiotic penicillin; industrial production of penicillin broadly classified in 2 processes, one is called upstream processing. Upstream processes encompass any technology that leads to the synthesis of product, then the upstream includes the exploration and development and production and downstream processing basically the extract, purification of biotechnology product from the fermentation is referred as a downstream processing.

So in a broader cells I can say that upstream processing we develop the technology through which we can produce in the penicillin in the fermentation broth and downstream processing we how this penicillin we can purify it from the fermentation broth. I told you that whenever we market any kind product it is necessary to purify the product if you do not purify then the value the cost of the product will drastically reduce, if more purification then the cost will be more high and it will be more useful.

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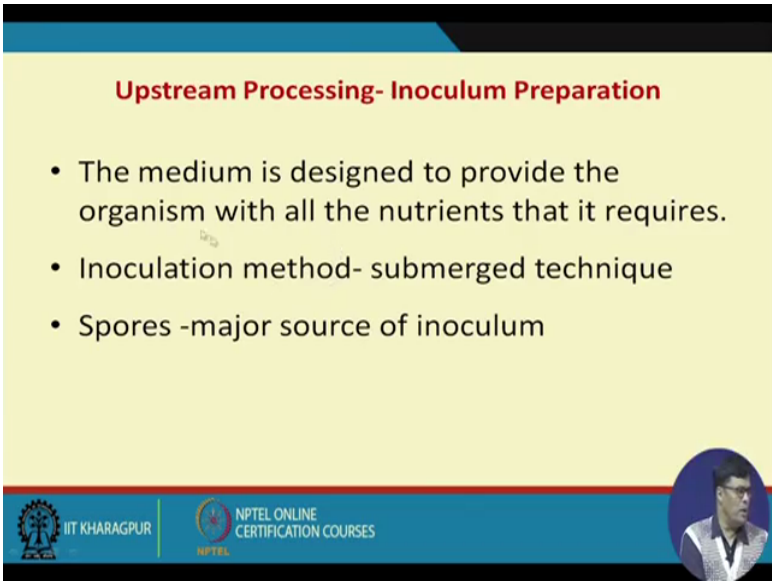

Then upstream processing the media is designed to provide the organism to provide the organism with the nutrient that is required, so this is very important. I told you not only the microorganism that is very important for the because microorganism that is used for the industrial fermentation process is to be should be the industrial strength. Industrial strength it is not only should have the higher productivity I mention that if the product concentration is high your recovery cost or purification cost will be low, so your cost of the product will be reduced drastically.

So this is one that is not another is the genetic stability, then biochemical activity of the organism should be same so that different generation you will get the same product. Productivity in if you use the different generation of organism that should not change with respect to generation. So this should be very-very this is very important factor that we have in case of penicillin or in any fermentation process.

Now media composition plays very important role for increasing the productivity of the system. So your media composition is to be optimised, so that we can get the good amount of this product formation. The inoculation method by submerged culture, I told you submerged culture is the best, the reason is that the organism can grow throughout the liquid. Spores are major source of inoculum, because in the lab if you prepare the culture is not that culture is not sufficient for the grow the culture in the production fermenter, so we shall have to prepare the spores. So quantify the culture and then we transfer it to the inoculum vessel where we

can get the vegetative cells, that vegetative cell can be used as a inoculum the production fermenter.

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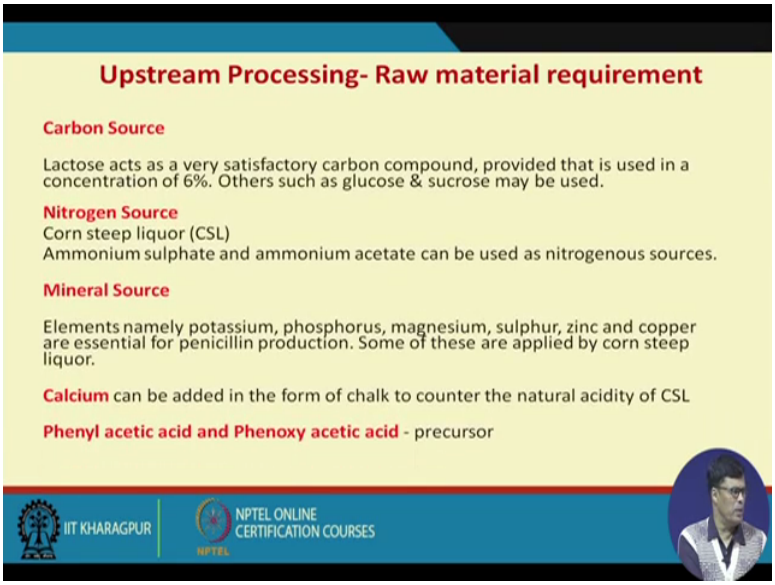

Now upstream processing, raw materials required maybe different. We have carbon source, we have lactose acts as a very satisfactory carbon compound, provided that is used in a concentration of 6 percent weight by volume, other such as glucose and sucrose may be used. Glucose and sucrose can be used as a energy source and lactose is used as a carbon source. Nitrogen source as I mention, corn steep liquor is appears to be the best carbon source as far penicillin production is concern the other nitrogen source are ammonium sulphate, ammonium acetate can be use also as a nitrogen source.

Different minerals, that also plays important role. Elements namely the potassium, phosphorus, magnesium, sulphur, zinc, copper are essential for the penicillin production. Some of these are applied by the corn steep liquor, because corn steep liquor I told you, corn steep liquor comprises of different of material because not only it contains nitrogen source, it contains some minerals, some kind of vitamins also it contains, so it is comprises of lot of things.

The calcium can be added in the form of chalk to encounter the natural acidity of corn steep liquor. Corn steep liquor has some kind of acidic pH, so we use the calcium, iron just to neutralise that. I told you the precursor is very much required for the penicillin fermentation process. The phenyl acetic acid and phenoxy acetic acid, phenyl acetic acid is used for the production of penicillin-G and phenoxy acetic acid the production of penicillin-V.

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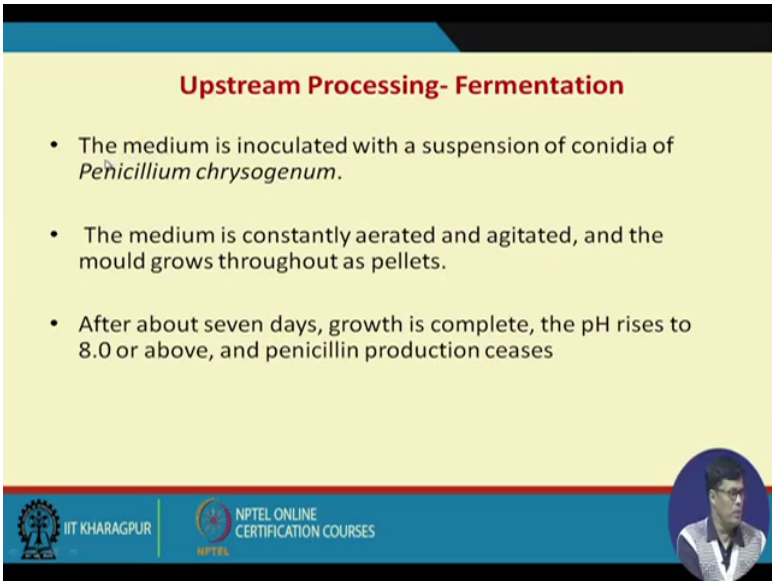

Now in the upstream processing we have other the media is inoculated with suspension of conidia that is that conidia spores actually that is penicillium crysogenum. The media is constantly aerated and agitated and the mould grows throughout as pellets. Now here again let me point out that in case of fungal fermentation process the we use the start tying reactor. Now in the start tying reactor we use the mechanical agitator, and it has been observed that if we put the agitator on the 0 hour it will affects the growth of the organism to a great extent.

So you have to keep the agitator off for some time, initial phase of fermentation let the mycelia to build up inside the reactor and then you put this. The main purpose of the agitator to keep the cell in the suspension but until and unless if they grow properly we cannot put it on, when it grow properly then and only then we put the agitator on make it suspension. So there is there are different ideal time for the production fermenter and for the inoculum vessel that is that varies from fermentation to fermentation process.

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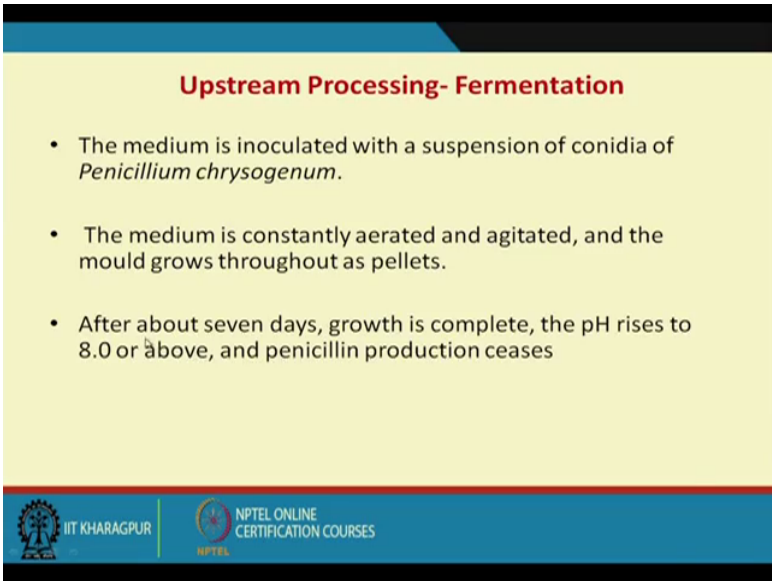

After about 7 days the growth is complete and pH rises to 8 and above and penicillin production ceases. Because now question comes how penicillin increases? Penicillin how the pH increases? PH increases due to reason mostly due to the deamination reaction and ammonia formation takes place from the protein. When undergo the deamination reaction is produce the ammonia and then ammonia formation increases the kind of pH of the media.

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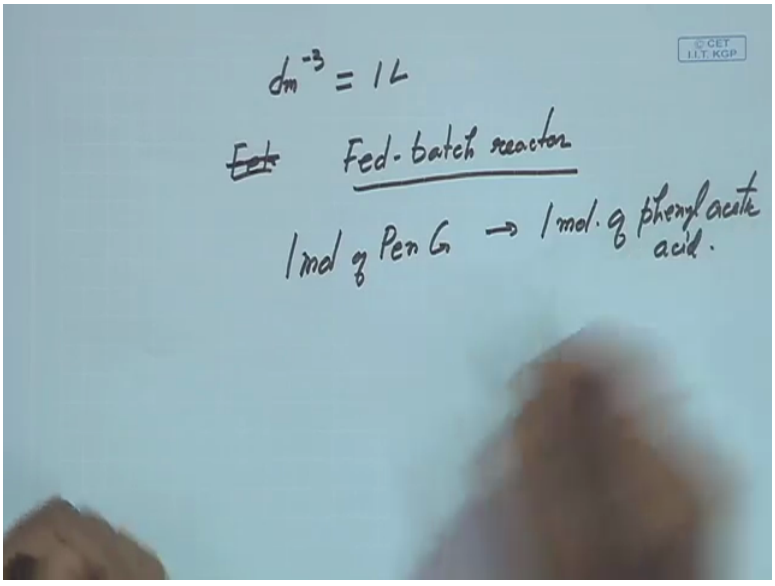

Now another thing I forgot to mention that fed batch reactor is appear to be best for this for the penicillin fed batch reactor is appear to be best in case of penicillin fermentation process, the reason is that we that phenyl acetic acid and phenoxy acetic acid is used as the precursor

and you know that stortumity is less, 1 mole of if you write 1 mole of penicillin that penicillin-G we require 1 mole of phenyl acetic acid.

So what I want to mention here that means you know how much penicillin is to be shown. If we on the basis of stortumity if we add all the phenyl acetic acid is the initial stage of fermentation process pH will immediately drop-down and that affects the fermentation process. That is why you have if you use the phenyl acetic acid in a fed batch way then and only then it will increase the penicillin production.

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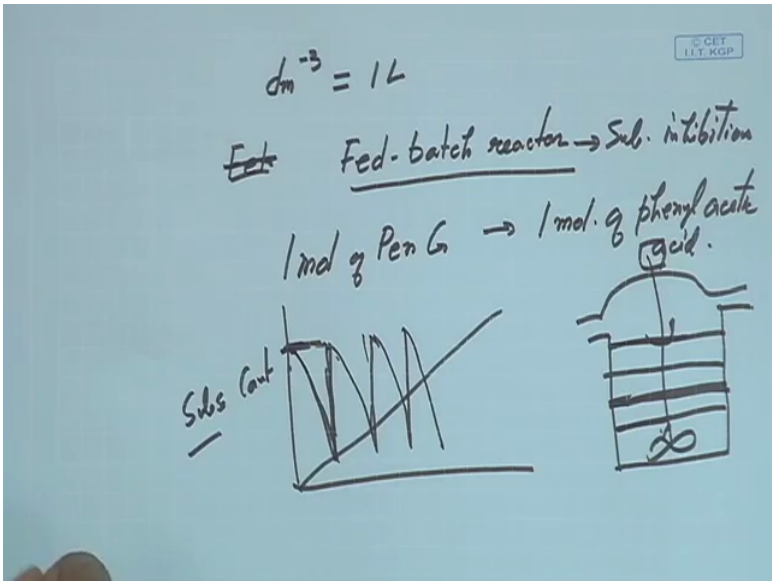

Then let me explain that the how the fed batch process works that you know that suppose there is a label that there is a you find out that substrate constant because this fed batch reactor is used in case of substrate inhibition in a in case of substrate inhibition it is used, suppose the substrate concentration that you find that this level is below the inhibition above this the inhibition takes place, so this concentration will keep on decreasing then again increase then again decrease then again increase like this.

So it is like this, this is a fermentation process, so you take small that media, that we have agitator here and then we with the concentration of the substrate will go down, then again we add another same volume of media, then again the concentration or you can increase little bit increase the volume like this and then so their concentration rise after this level. Again concentration will drop-down and again you increase the concentration like this again it is drop-down again you will the concentration level. So like this you come here when it is totally filled up then use to when use to your fermentation complete, your product formation

keep on rising like this, so when then you stop fermentation and take out the product from the reactor.

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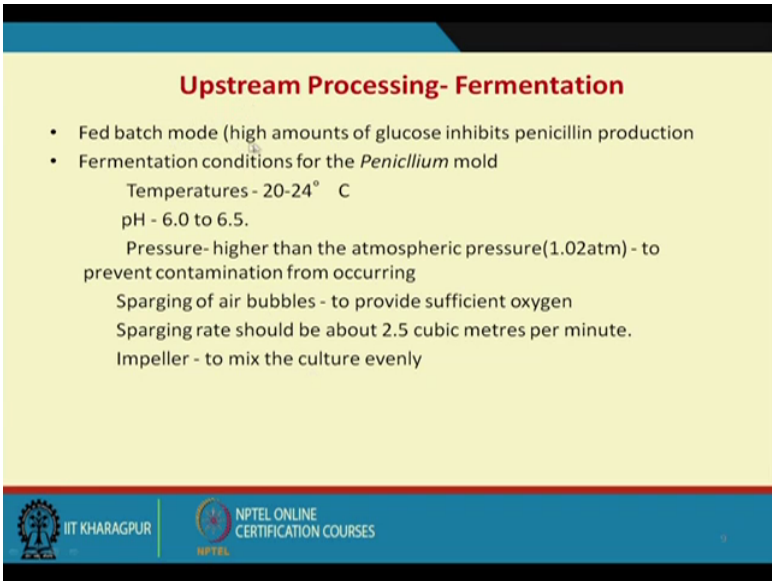

So this reactor fed batch reactor was found most suitable for the penicillin fermentation process because substrate here substrate acts as a inhibitor. After that is after the end of the fermentation pH is rises to 8. Fed batch mode high glucose inhibition of penicillin production takes place that can be taken care by using the fed batch reactor. The fermentation condition is 20 to 24 degree centigrade, pH 6 to 6.5, pressure higher than the atmospheric pressure 1.02 to prevent the contamination.

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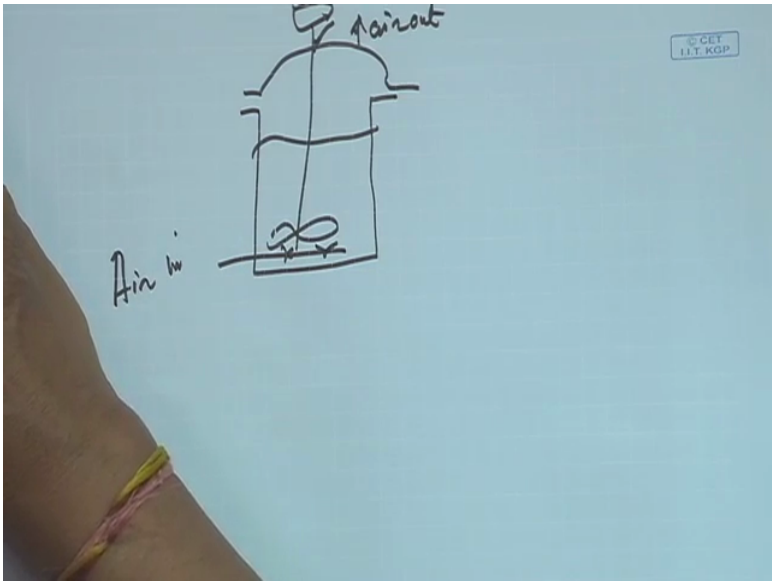

Because here let me tell you the very interesting thing that this is a aerobic fermentation process. So when you do the aeration all is there will be your air is going out, air-out and air-in. So if they we have more air there will be some positive pressure if there is a positive pressure then there is less possibility to enter the air from different sources, because even there is a leak in the in case of mechanical seal that your air will not come, because if it is a positive pressure the air contamination problem can be avoided.

That is why it is written there, higher than the atmospheric pressure to prevent the contamination from occurring. And sparging of air bubbles to provide the sufficient oxygen, because oxygen is sparingly soluble in water and sparging rate is about 2.5 cubic meter per minute, it depends on the volume of the fermenter.

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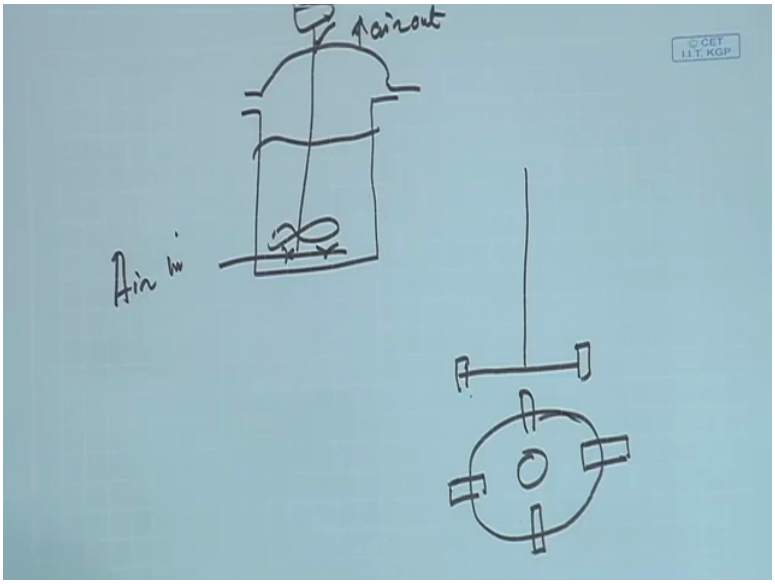

Impeller to mix the culture evenly, so it is the use the impeller, impeller I have already shown you how it looks the impeller it is like this and if you take that you know the top view or bottom view it is like this, the disc, in the disc this is centre point and the this is the blades are mounted like this, so this is rotate like this and this is called impellers.

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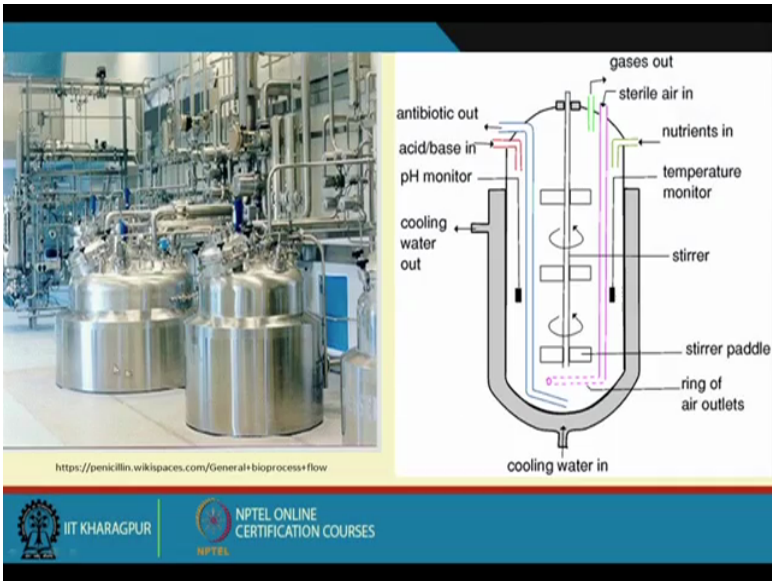

And this is the fermenters and this how the impellers **(())(019:21)** looks. There is the different type of fermenter that is used we have we can have this in different air in I showed you there is the air-in and this is air-in and air- out, this is that we have. Air-in here and air–out gas-out that is temperature monitoring system that we have.

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Now after the fermentation is over we shall have to purify the product which is very important. Let me give you some detail information on the purification process. Now downstream process is relatively easy since the penicillin secreted in to the media and this is the extracellular product this is not a intercellular product, so there is no need of breakup break open the fungal cells. So however the product need to be very pure and since it is being

used for therapeutic medical drug, so it is dissolved and then precipitated with potassium salt separate it from other substances in the media.

So I told you by solvent-solvent extraction process the penicillin is purified and then finally we add some kind of sodium or potassium salt to produce the sodium and potassium salt of penicillin. And then removal of cells, first steps is the removal of cells that recover in the separation of the whole cells and other insoluble ingredients of the culture broth that is the filtration we use.

We use the rotary vacuum filter. I already mention what is called rotary vacuum filter, we have a drum and inside that it is inhibited with thin muslin cloth and one knife that touches on the surface of the muslin cloth and it is immersed in a tub where your fermentation broth is there and we apply vacuum in the pipeline so that it sucks the water, suck the water and cell, suck the liquid and the cell with a with stick on the surface of the muslin cloth and since when it is touching with the muslin cloth surface the cell automatically will be dislodge from the surface of the that of the muslin cloth and we can collect it in the collector or in the wagon relay wagon we can collect for take it out.

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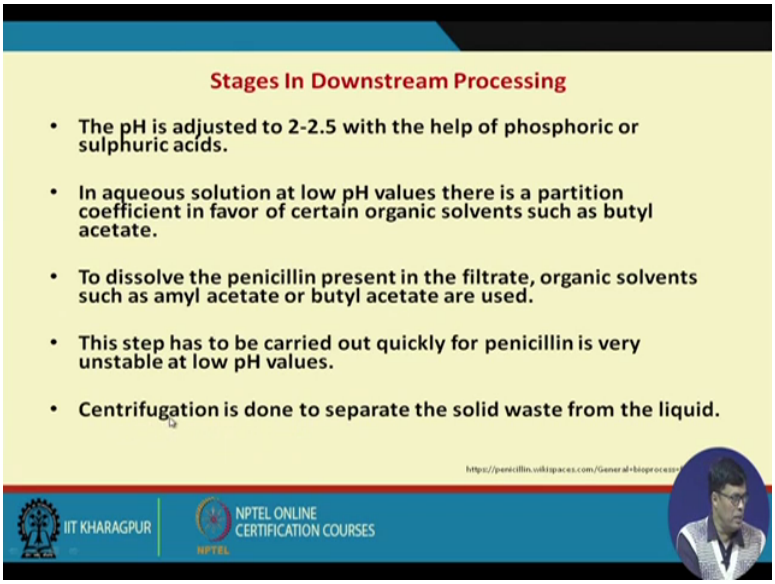

Now after taking out this as I told you, the pH is adjusted to 2 to 2.5 with the help of phosphoric and sulphuric acid and then in the aqueous solution at low pH there is the partition coefficient in favour of certain organic solvent such as the butyl acetate, amyl acetate. This they show as you when you reduced the pH, the partition coefficient will be that is solubility of solute will be more in butyl acetate as compared to the aqueous layer.

So most of the penicillin that present in the aqueous layer that goes to the solvent layer and to dissolve the penicillin present in the filtrate the organic solvent such as acetyl butate are used. Then these steps has to be carry out quickly for penicillin is very unstable at low pH, because we know penicillin particular penicillin-G it is very unstable at lower pH. And then centrifuged is done to separate the solid waste from the liquid.

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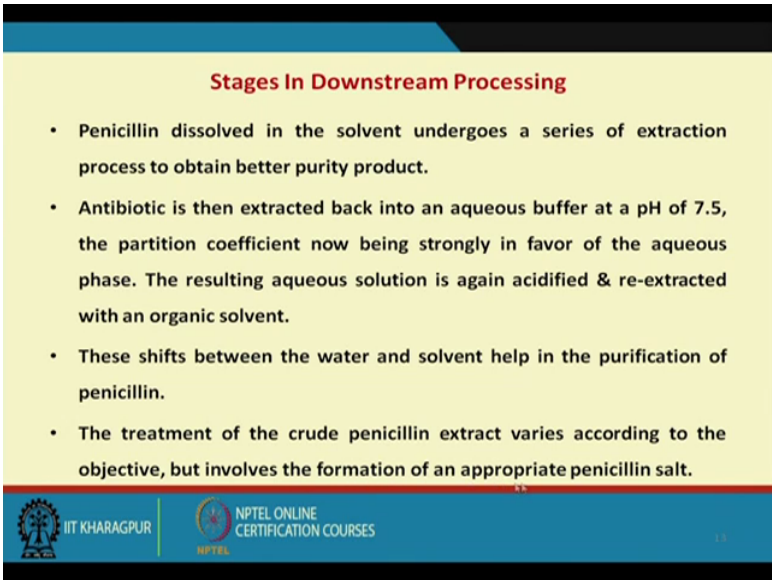

Penicillin dissolved in the solvent undergoes series of extraction process to obtain the better purity, because again we increase the pH of the some aqueous solution we use some kind of solution, so that there is kind of buffer solution, so that penicillin can comes to the aqueous layer again it goes to the solvent layer like this we have the purification of the process. Antibiotic is then extracted back to the aqueous buffer at pH 7.5, the partition coefficient now being stronger in favour of aqueous phase.

The resulting aqueous phase solution again acidified and re-extracted with the organic solvent. So this is kind of you know repetition of the process that is going on to get the purification product. This shifts between water and solvent help the purification of penicillin. The treatment of crude penicillin extract varies according to the objectives, but involves the formation of appropriate penicillin salt.

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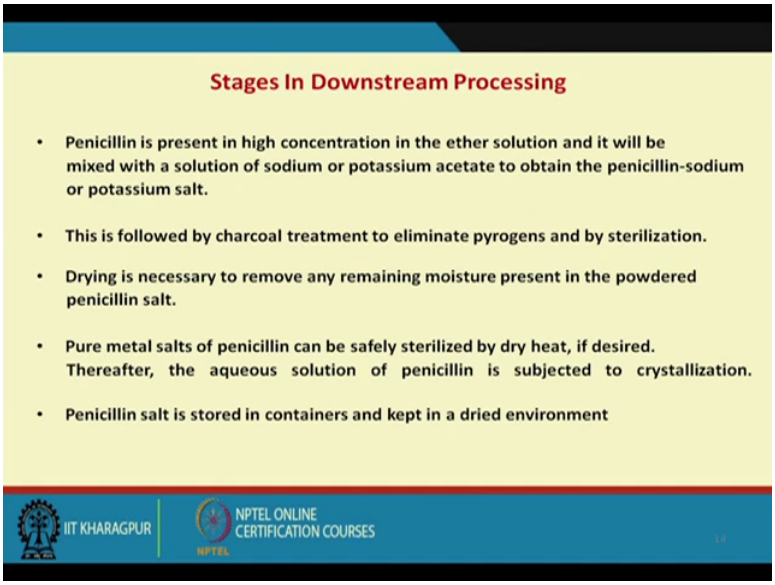

And penicillin is present in high concentration in the ether solution is being mixed with a solution of potassium or sodium acetate to obtain the penicillin or sodium salt of penicillin. So you know we can penicillin-sodium or potassium salt we can produce if we use the sodium salt or penicillin potassium salt then we get that. This followed by charcoal treatment, because we have seen that charcoal have some bleaching this eliminate the pyrogen and by sterilisation process.

And drying is necessary to remove the remaining moisture present in the powdered penicillin salt and pure metal salt of penicillin can be safely sterilised by dry heat, if desired and therefore in the aqueous solution of penicillin is subjected to crystallisation. Penicillin stored penicillin is stored in a container and kept in the dry environment. So this is the I told you that that particularly penicillin when it is used in the form of capsule it is not required that much of stinging condition but when you use in the form of injection vials it should be 100 percent sterilised, but since it is a it is used for the therapeutic purpose it should be as pure as possible.

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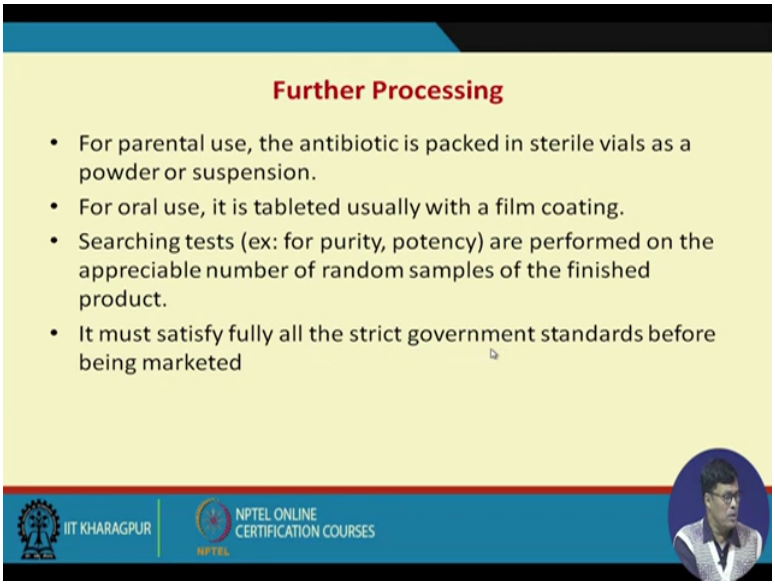

The further processing for parental use, the antibiotic is packed in a sterile vial as a powder suspension. For oral use, it is tableted using the film coating. Then searching test for purity potency are performed on appreciable on the appreciable number on random sampling of the finished product. And it must satisfy fully the strict government standards before being marketed.

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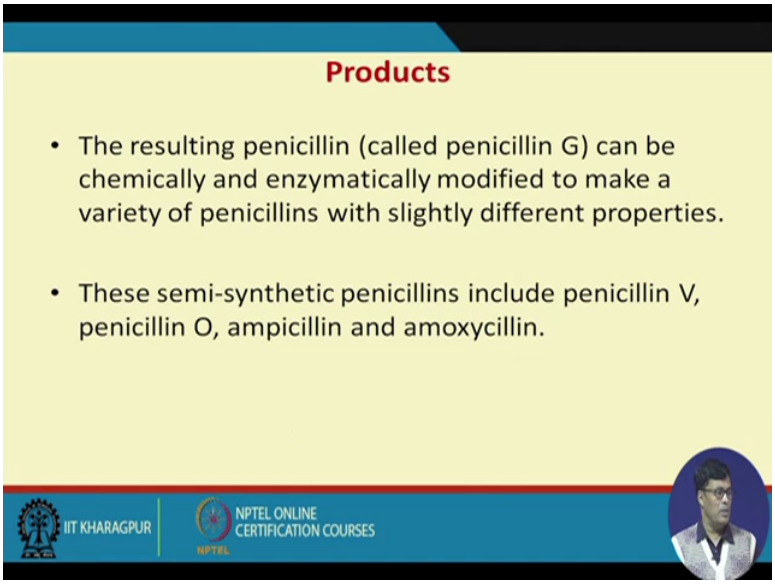

So products, the resulting penicillin can be chemically and enzymatically modified to make the varieties of penicillin with slightly differs in property, because as I mention that penicillin-G is unstable in the acidic pH but when it produce the penicillin is converted to the ampicillin, this is the this is little bit stable in the acidic pH as compared to the penicillin-G.

This semisynthetic penicillin includes the penicillin-V, penicillin-O, ampicillin and amoxicillin.

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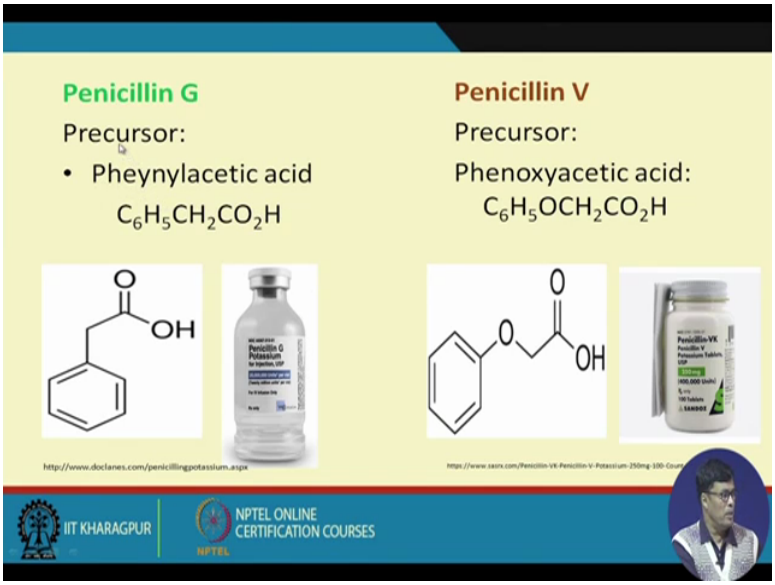

Now precursor I told you that penicillin-G we have phenoxy acetic acid formula is this is sorry this is phenyl acetic acid C6H5CH2 the acetic acid formula is CH3 C double O H so 1 hydrogen will be removed and it is C6H5CH2 C double O H this is phenyl acetic acid from that we produce the penicillin-G and this is potassium salt of penicillin mostly used in the form of injection vial and sodium salt actually that is used for maybe in the form of capsule that is preferred we use the phenoxy acetic acid this is use in the form of tablet this is penicillin-V. And the phenoxy the way you O-group is there, this CH2 but this is O-group is there that is OH group is there that is why we call it as phenoxy this is used for the production of penicillin-V.

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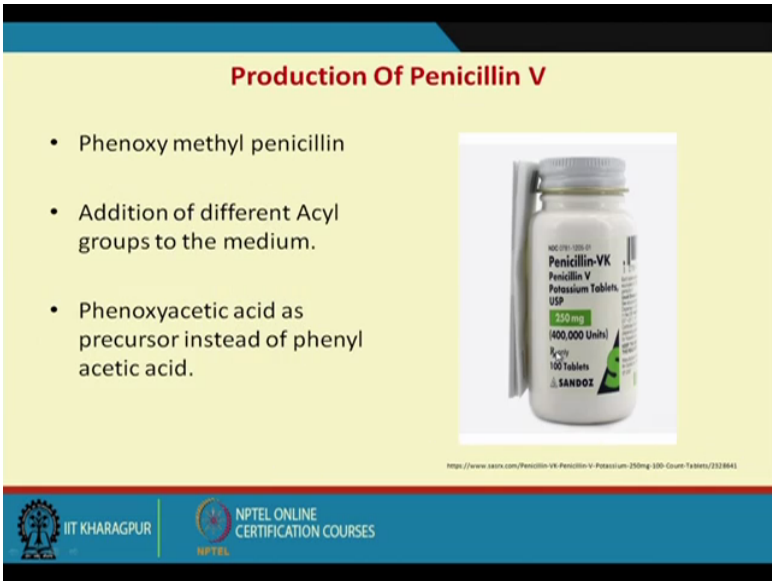

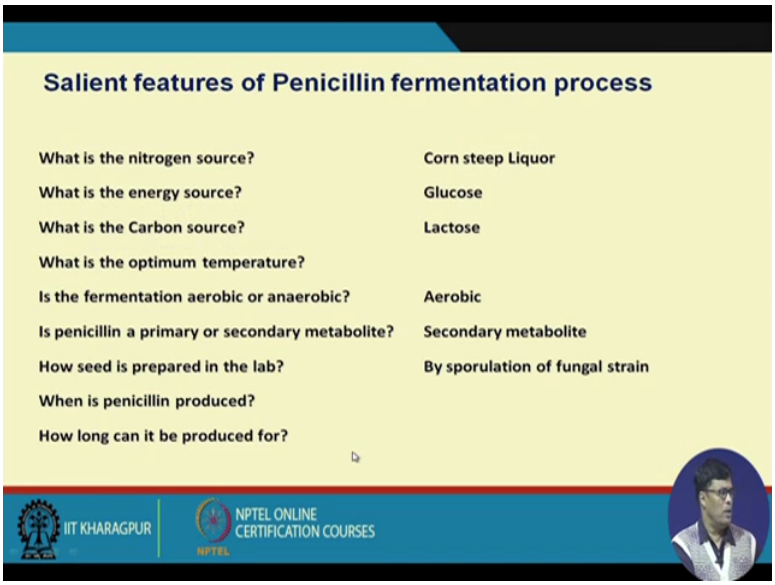

Now the phenoxy methyl penicillin the production of penicillin-V is the phenoxy methyl penicillin, addition of different acyl groups to the media and phenoxy acetic group is the precursor instead of phenyl acetic acid that is we use. Now there is some silent features we have in the penicillin fermentation process, I like to mention, that nitrogen source is corn steep liquor, then energy source is glucose, carbon source is lactose, optimum temperature is 25 to 27 degree centigrade, the fermentation is aerobic, then it is a secondary metabolite product, that how the seed is prepared? By sporulation of the fungal strain, but this sporulation we do not use for the production fermenter, this we produce they inoculated in the inoculum vessel to get the vegetative cell and that vegetative cells is used for a as inoculum for the production fermenter.

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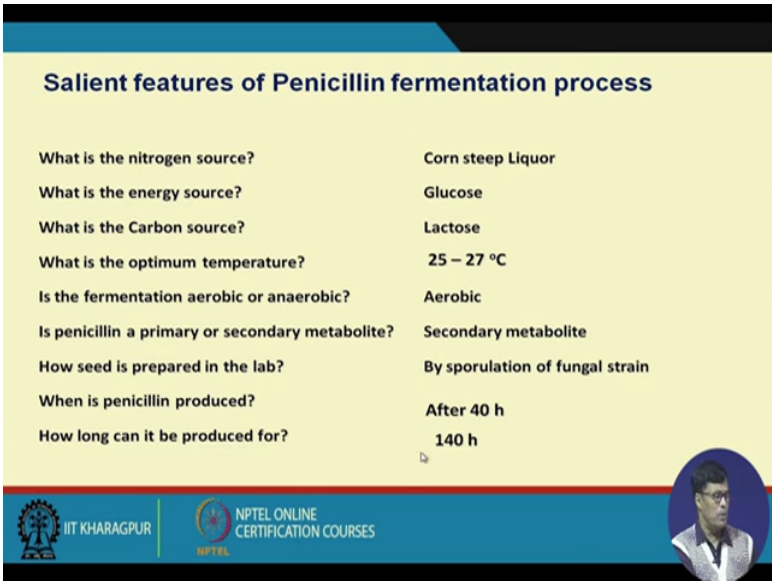

Then when the penicillin is produced? After 40 hours of fermentation and how long this production takes places? That is 140 hours. What is the precursor of penicillin-G? That is phenyl acetic acid. Now if I asked you what is precursor of penicillin-V? Phenoxy acetic acid. What was the first fungus known to produce penicillin? That is penicillin notatum. Then what is the species produce 6 milligram per litre of penicillin? This is penicillin Crysogenum. What is the how did the scientist improve the yield still further? By Genetic modification.

So what is the batch culture is used? What is the why batch culture is used? The problem of aeration that is why is used. And major problem of penicillin fermentation is substrate inhibition, and to improve the substrate inhibition to take care of substrate inhibition we can go for the fed batch process where the substrate inhibition can be we taken into account.

And then how what are the major downstream processing that we have? Liquid-liquid extraction, butylacetate or amyl acetate that we use, precipitate of potassium salt and aseptic condition. Why cannot the penicillin-G be taken orally? Because it is unstable in acidic pH. Name the form of penicillin which can be taken orally? That is penicillin-V or ampicillin. How the penicillin does killed the bacteria? To stop the production of cell wall and why the gram negative bacteria not killed by the penicillin? Because, it has the different cell wall.

So we can understand that different use of penicillin how the penicillin act on the human body, how the production of penicillin takes place, which are several we have huge market in the biochemical industries and we are greatly benefited due to the due to penicillin that is which produces in the market available in the market and this has regular use for curing the

different infections particularly in the in case of athletics it is quite regular because they might be having some wounds in the when they fall down that causes some kind of pusses and this usually taken care if you use some kind of penicillin. And penicillin can be used largely used for different type of curing the different type of infections.

So with this I want to point out that if you look at the extraction process that if you compare the downstream processing as compared to citric acid production, citric acid production also takes place by using fungi and penicillin also produce by using the fungi. In case of citric acid we use Aspergillus Niger, penicillin we produce by penicillium crysogenum, both are fungi. But if you look at both are secondary metabolite's and but if you look at the downstream processing there are a lot of difference in the downstream processing of citric acid we separated through the process of crystallisation then here we separate the penicillin by solvent-solvent extraction process.

After that we not we use some kind of sodium or potassium salt of salt, so that the acetic acid so acetate to get the sodium and potassium salt of penicillin and finally we can produce in the form of crystals, dry powder or injection fluids. I hope this information will be good enough to understand about the penicillin fermentation process. Thank you.