Course on Industrial Biotechnology
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Module 08
Lecture No 39
Penicillin Production

Welcome back to industrial biotechnology course. Now in the last lecture I try to discuss the glutamic acid fermentation process, this kind of amino acid production, so we tried gives some kind of information that how the amino acid can be produced. Now in this lecture I am starting with new areas, that is production penicillin, because penicillin is considered is the first antibiotics that is ever produced and that used for curing the wounds, particularly we have during some accident and other thing.

So that wound is mainly due to the infection of gram positive bacteria. So with the intention of this penicillin we can nowadays we can cure the disease. Particularly in case of World War 2 it was very much required, because lot of soldiers was wounded and they required this kind of antibiotics for curing their wounds.

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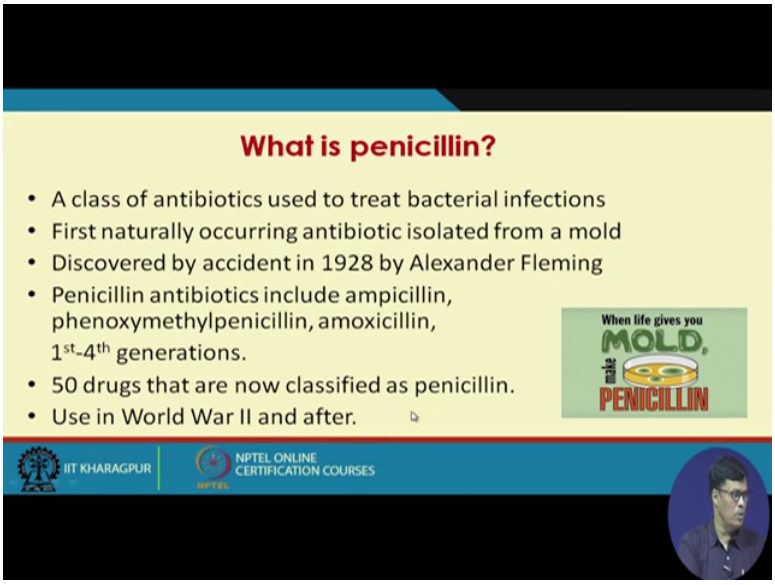

So let me go ahead with this, and first question that is arises that what is called penicillin? Penicillin is a kind of antibiotics used to treat bacterial infection. Because antibiotics let me tell you antibiotic is has a bit kill the germs actually, because it is kind of infections that we have it kills that, that is antibiotics. And first naturally occurring antibiotic isolated from the mould, so the mould is kind fungi that is used for the production of this antibiotics and discovered by accident 1928 by Alexander Fleming. So this is a very famous scientist first discovered this penicillin which has some antibacterial property.

The penicillin antibiotics includes the ampicillin, the phenoxymethylpenicillin, amoxicillin, then first to 2nd 4th generation antibiotics that we produced from the penicillin. 50 drugs that are now classified as penicillin. The penicillin I shall show you the speciality of the penicillin it has the Beta-Lactam ring, so that we had we can produce 50 different drugs that classified as penicillin, so used in World War 2 and after. So as I mentioned this is after that this is used.

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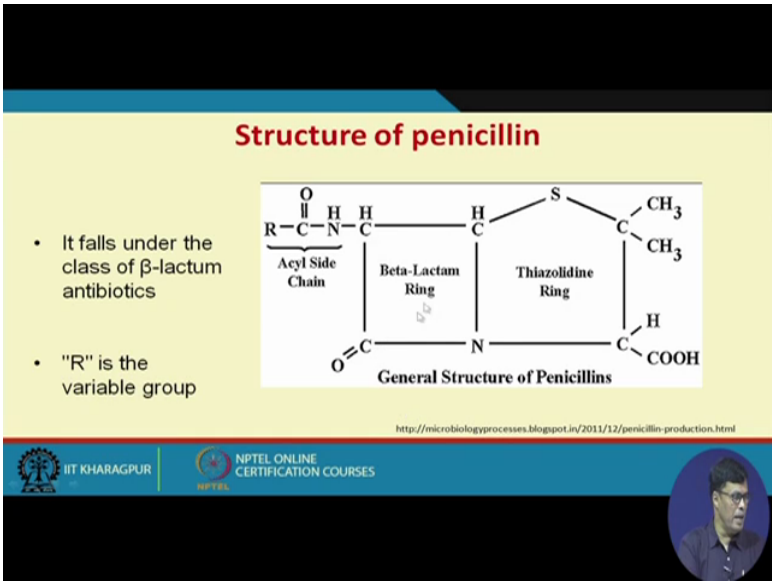

Now this is as I told you that this is the penicillin the speciality is that they have this Beta-Lactam Ring that we have it falls under the class of Beta-Lactam antibiotics. So this is called the Beta-Lactam antibiotics and this is the Thiazolidine this ring that we have. So most of antibiotics this portion is remaining constant only the Acyl side chain this will keep on changing, because this is the R group that keep on changing that.

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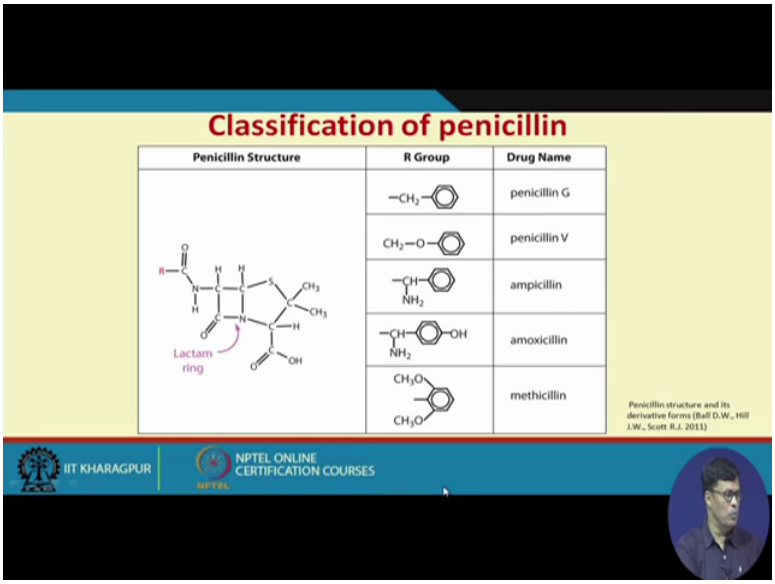

Then let me show you this, this is Beta-Lactam and this is the R group and this R group might be different. We have if R group is this methyl benzene or phenyl benzene, methyl benzene then we because this is precursor is the what you called phenyl acidic acid, we have phenoxyacetic acid that we have different type of R-group, if present there we have different

types of antibiotics but all are penicillin group, because all has the Beta-Lactam this the ring that we have. So this is penicillin-G, penicillin-V, ampicillin, amoxicillin and methicillin.

Now largely we use this penicillin-G and penicillin-V, because from penicillin-G we produce ampicillin and other antibiotics. Penicillin-V, the basic difference between the penicillin-G and penicillin-V is that, penicillin-G is quite unstable and acidic pH, so we know that penicillin usually taken in 2 different forms, either in the form of capsule or either in the form or in the form of injection fluid.

Now if it is injection fluid, our blood pH is almost close to the neutral so if you take penicillin-G when the fluid do not have any problem, but take as a capsule our stomach pH is about 2, so it is quite unstable at the pH 2 so activity of the penicillin will be lost. So we find comparatively penicillin-G is better, so penicillin-G is usually taken in the form of penicillin capsule.

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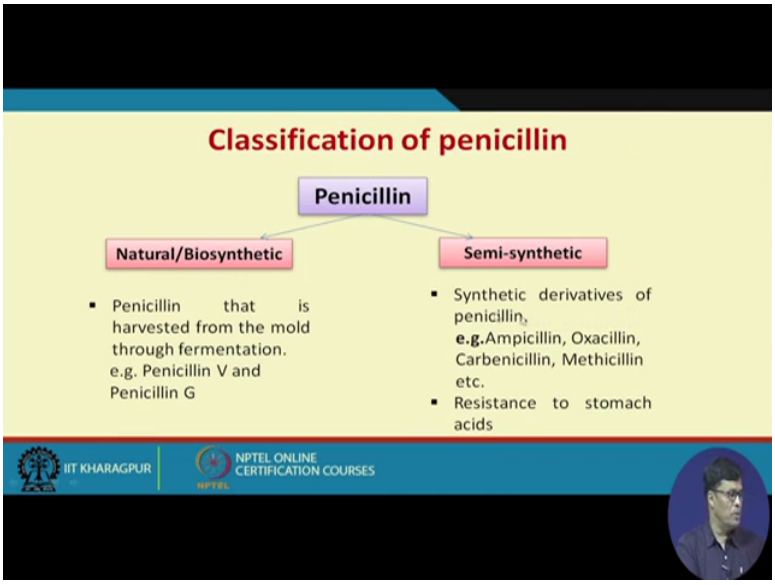

So we have the classification of penicillin, we have natural or biosynthetic way. We can penicillin that is harvested from the mould through the fermentation we considered as a natural or biosynthetic penicillin-V, penicillin-G. And semi-synthetic, semi-synthetic derived from the penicillin because we add some kind of we make some kind of chemical alteration here to get the ampicillin, oxacillin, carbenucillin and methicillin etcetera, resistant to stomach acid. And this penicillin, they are resistant to stomach acid we can take it in the form of capsule because then it will be quite (())(06:22) that is not natural not pure natural but

semi-synthetic. Semi-synthetic means this is derived from the penicillin which is mostly it is derived from the penicillin-G that is it is.

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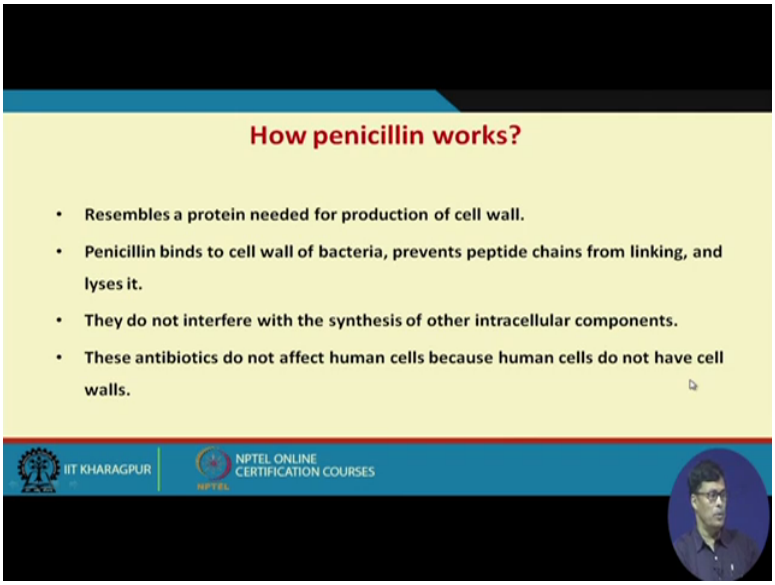

So now question comes how the penicillin acts on the this organism which is pathogenic which causes the wounds, because very interesting. It is resemblance proteins needed to the cell wall, so its structure is quite similar with the protein needed for the cell wall formation. Penicillin binds with the cell wall of bacteria and prevents the peptide chain from linking and lyses it. So lyses it is you know that it is kill it is just destroyed that and they do not interfere with the synthesis of other intracellular components, because they inhibit for the main purpose of the penicillin they inhibit the cell wall formation in the bacteria.

So since the cell wall formation of the bacteria is inhibited then cell wall will be killed. That is how the penicillin works, but it does not affect the intercellular component, because when you take in our system it will affect the bacteria, but it will not affect the intercellular component of our system, so that is very important. These antibiotics will not affect human cells because human cells do not have the cell wall.

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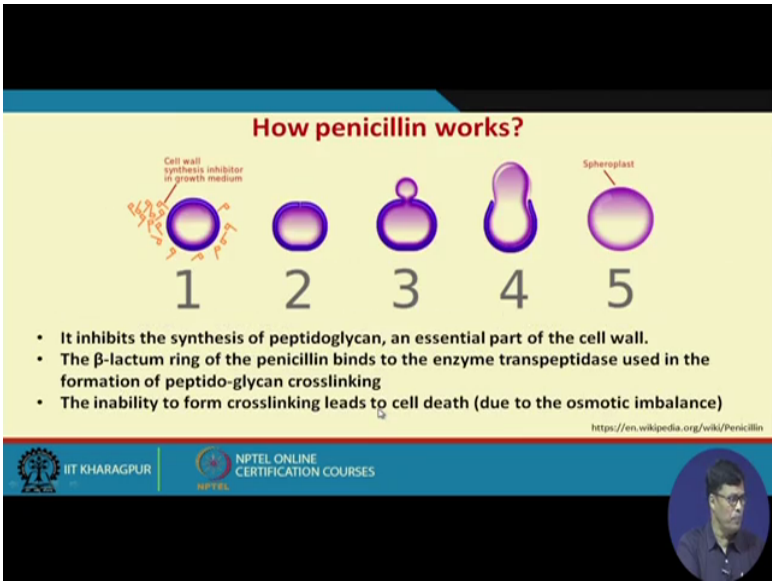

Now this is how the penicillin works, it this is you can see how this is the cell wall synthesis inhibited in a growth media. This is the inhibitor that we have that inhibits the synthesis of peptidoglycan, an essential part of the cell wall. The Beta-Lactam Ring of the penicillin binds to the enzyme transpeptidase used in the formation peptide-glycan cross-linking. And then the inability to form the cross-linking leads to cell death, that this is the reason why the cell death occurs, due to osmotic imbalance. As soon as the cell wall has been removed, then what will happen, there will be some osmotic shock in the microorganism, then the microorganism will be killed. This is because the cell wall is very important component of the microorganism.

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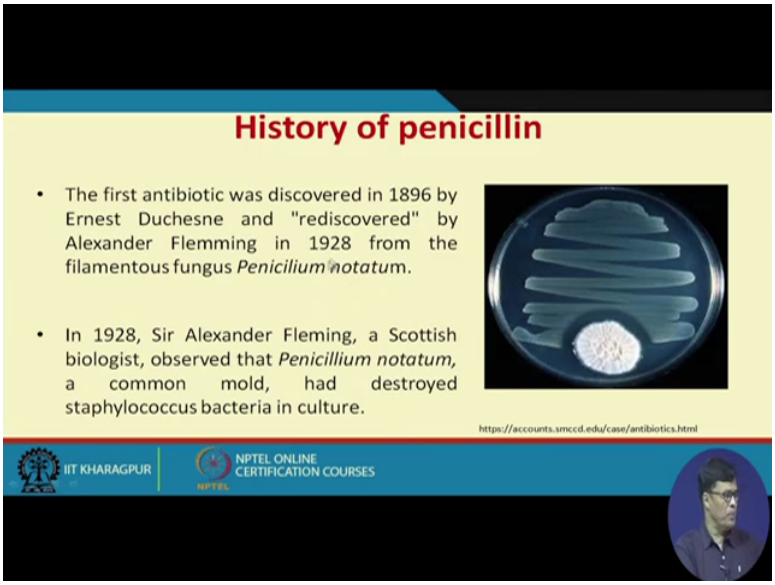

Now let us talk about the little bit about the history of penicillin, the first antibiotic was discovered in the year 1896 by the Ernest Duchesne and rediscovered by Alexander Fleming in 1928 from the filamentous fungus penicillin notatum. So nowadays we produce penicillin by penicilium gricious, I shall discuss how the transformation has been taken place. The first penicillin that has been identified for the formation of penicillin that is penicilium notatum and this is how it grow on the surface of the platiplates and the fungal looks like this that whatever is shown in the on the platiplates.

In 1928 Sir Alexander Fleming a Scottish biologist observed that penicilium notatum a common mould has destroyed staphylococcus bacteria in culture. So Staphylococcus we know it is very harmful bacteria for us and he found out that the Staphylococcus growth of Staphylococcus will be is greatly interfere greatly inhibited in presence of this particular mould which produces the penicillin. It was further discovered by the Alexander Fleming like this.

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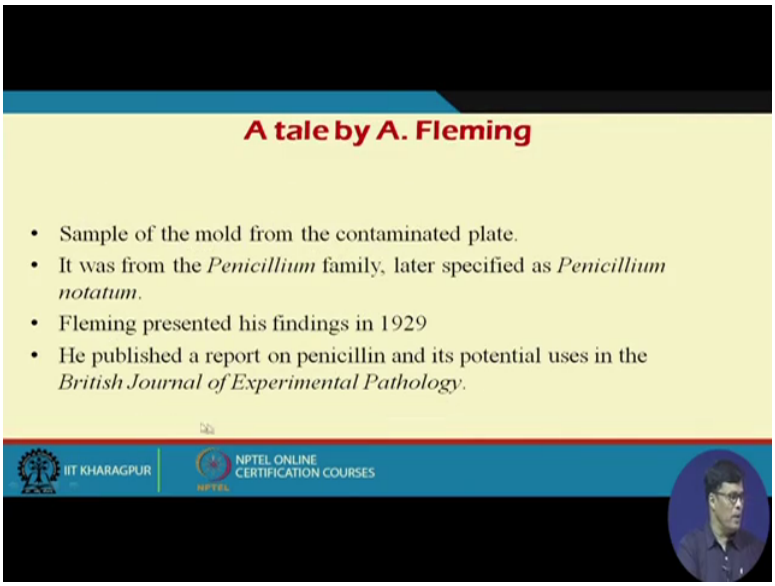

And then the sample of mould from the contaminated plate is taken out, it was penicillin family and later identified as penicilium notatum. Later he identified the particular organism what is the organism from the plate and he identified the organism as penicilium notatum. The penicillin the Fleming presented his findings in 1928. He published the report on penicillin and its potential use British Journal of Experimental Pathology.

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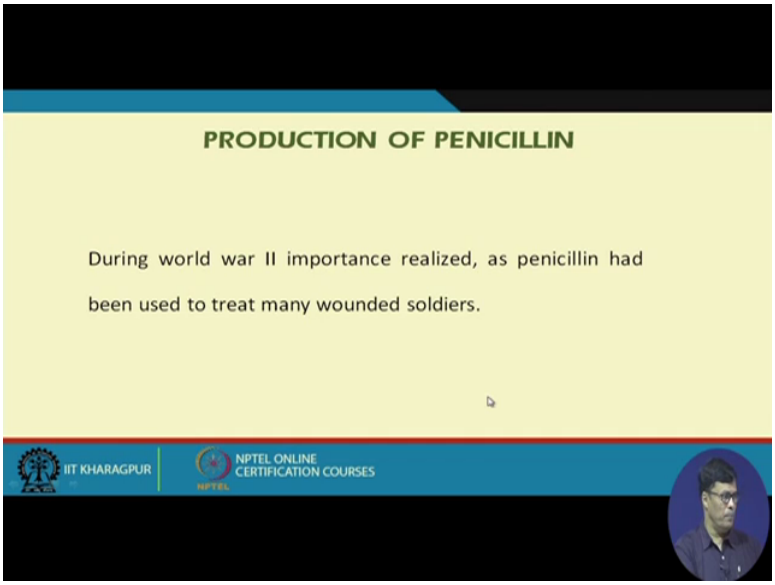

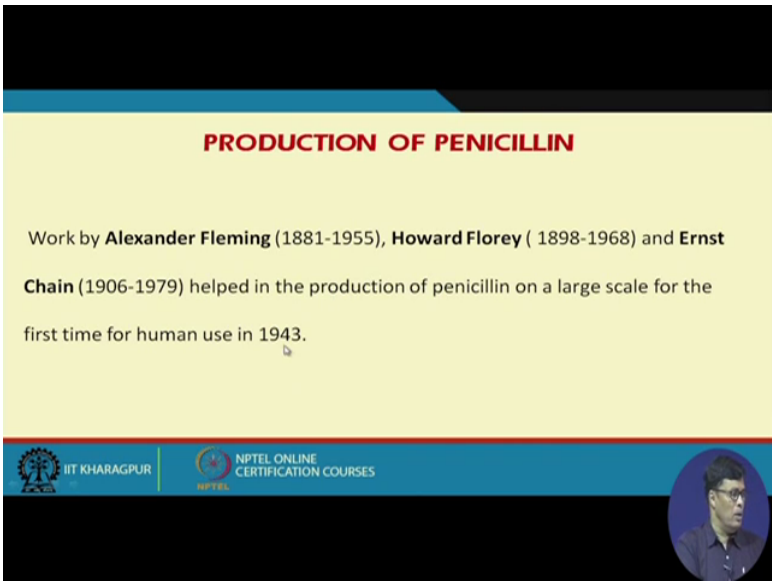

Now production of penicillin during World War 2 importance realised, as penicillin has been used to treat the many wounded this soldiers, because I told you before also this importance of the penicillin that visualises after the World War second. Work of the production of penicillin work by Alexander Fleming 1981 to 19 1881-1955, Howrad Florey 1898-1968 and Ernst chain 1906-1979, help in the production of penicillin on a large-scale for the first time for human consumption that human use 1943. Now here I want to point out in India Sara Bhai Chemicals that is one company that produced penicillin through this fermentation process and they use the penicilium gracious crysogenum for the production of penicillin.

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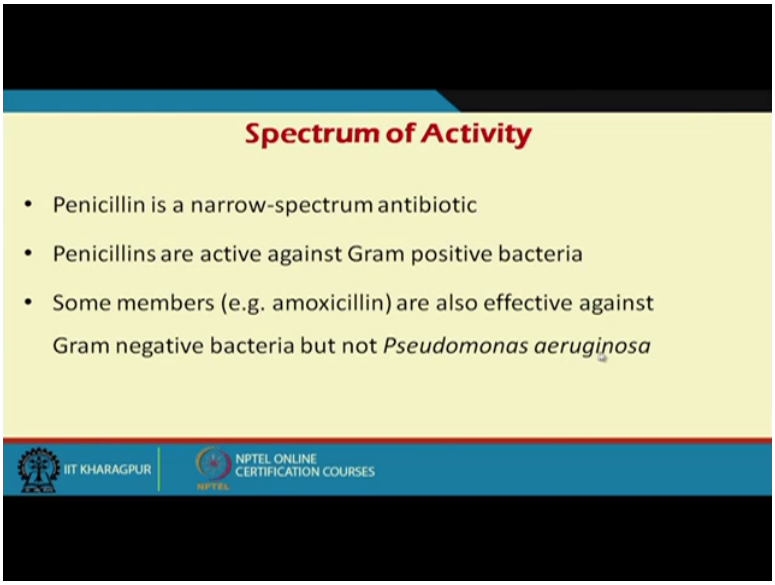

So now spectrum activity of penicillin, penicillin is considered as a narrow spectrum antibiotic. You know that antibiotics has 2 types of antibiotics, one is narrow-spectrum and another is wide-spectrum antibiotics. Narrow spectrum antibiotics means they are very specific against any bacterial species and wide spectrum antibiotic means they can act against the different type of bacteria.

So that is the as for example the penicillin is considered as the narrow spectrum antibiotic but streptomycin is considered is a broad spectrum antibiotic. And penicillin only can act on the gram positive bacteria and then some member like amoxicillin because which you drive from the penicillin are also effective against the gram negative bacteria but not pseudomonas aeruginosa.

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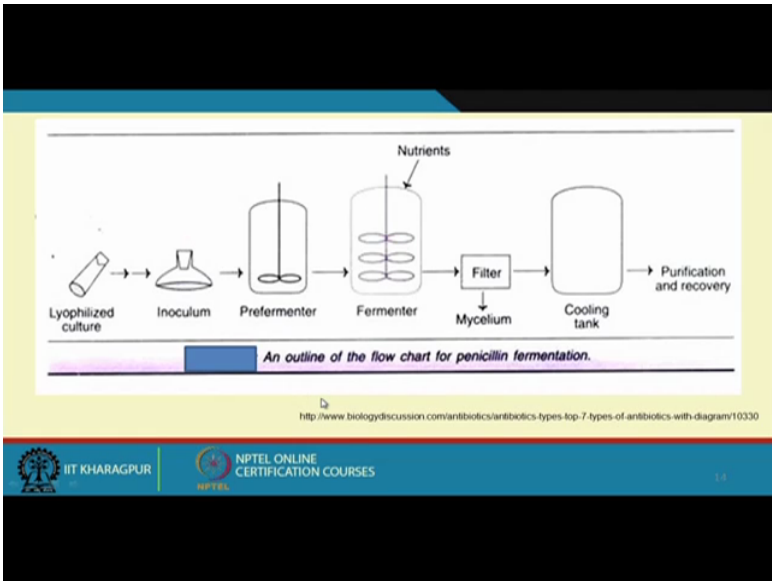

The production of penicillin, let me discuss how the penicillin production takes place it is very it is common as compared to the particularly citric acid fermentation process you see that we have the lyophilized culture, then we know that how to activate the lyophilized culture. I told you that culture may be available in 2 different forms, either in the form of lyophilized culture or in the form of slant culture.

The first we have to activate the lyophilized culture and put it in the slant because how it is done, it is very simple procedure that we have. We know the lyophilisation process I have I already discussed, lyophilisation is the phiz drawing process under present condition this is moisture is taken out water molecule from the solid to vapour phase is taken out, so that your microorganism can be preserved for a longer period of time.

These dry powder which contains the cell which can preserve for couple of years we can take it out and then we can we make a suspension in 0.85 percent saline water just we can make a suspension. Then we can take one spoon and one loop full of this culture and then we stick on the particular slant. You know that agar slant, we can have the media we can put the scratch on the surface.

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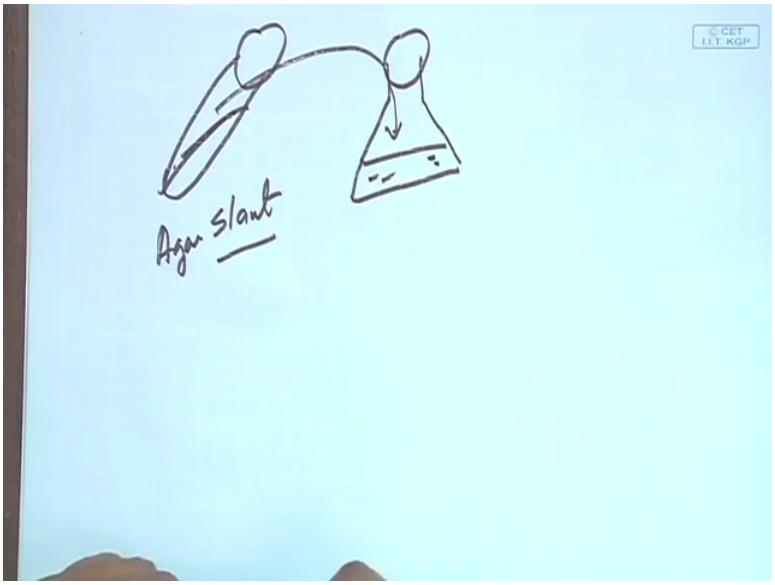

So while we produce this slant, this is slant and then agar slant and then we this agar slant then we from this we keep it in the incubator, allow the cell mass to grow, after that we take this culture we transfer to the liquid media which is in the conical flask. Conical flask we have liquid media, so this is we from this we this culture will transfer here when grow we transfer here.

So here what is then we take it in the inoculum vessel, we increase the volume and finally we put it in the inoculum vessel. I told you the volume of the inoculum vessel is 10 to 5 to 10 percent as compared to fermenter. Now here I want to point out that in case similar to the citric acid fermentation process that we use that aspergillus niger which is also fungi and here we use penicilium glycogenom which is also fungi.

So what is happening, that we shall have this fungi is a filamentous organism. So I shall show you the structure or morphology of this particular organism. That what we will observe that this organism we cannot count, so what you have to do, we shall have to put the do the sporulation of this microorganism and sporulation I have already shown you how the sporulation is done. I mention that by using the weighted this barley grains and honey peptone media it is possible to produce the spores of the any kind of the fungal cells.

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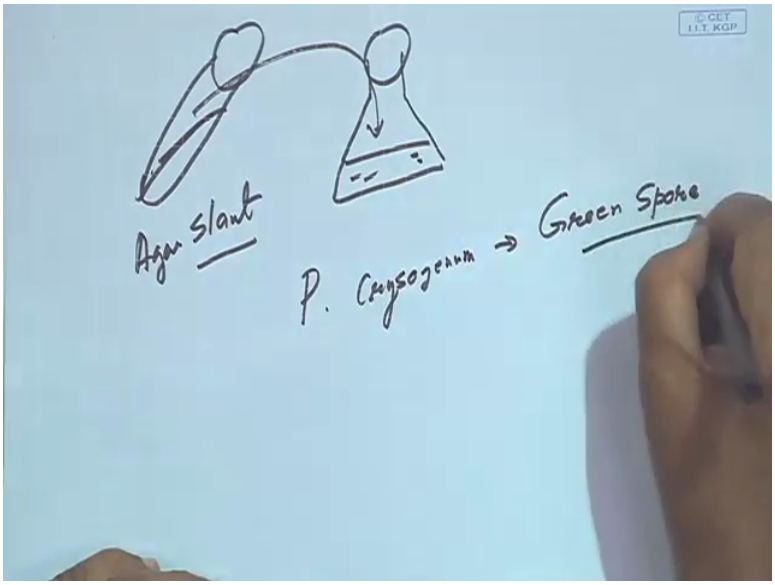

So and here I want to mention that penicilium crysogenum this is has the green spore, green spore culture that you know that in case of this what you called in case of aspergillus niger we have the black spores and here we have the green spore and we can count it and we can prepare the spores with spurious culture in the lab and this culture we can we take it in the seed can and from the seed can we transferred it to the inoculum vessel this is what you called as a pre-fermenter.

And now here we produce the vegetative cells and these vegetative cells you put it in the production fermenter. And after production fermenter, first you passed through the filter, filter means Rotary vacuum filter because Rotary vacuum filter already explained since the fungus fungi fungus size are quite big couple of millimetres, so it can be easily separated with the help of Rotary vacuum filter where you can get the mycelium and this can be mycelium can be you for the production of rough quality paper then you put it in the cool tank and then to the purification and recovery process.

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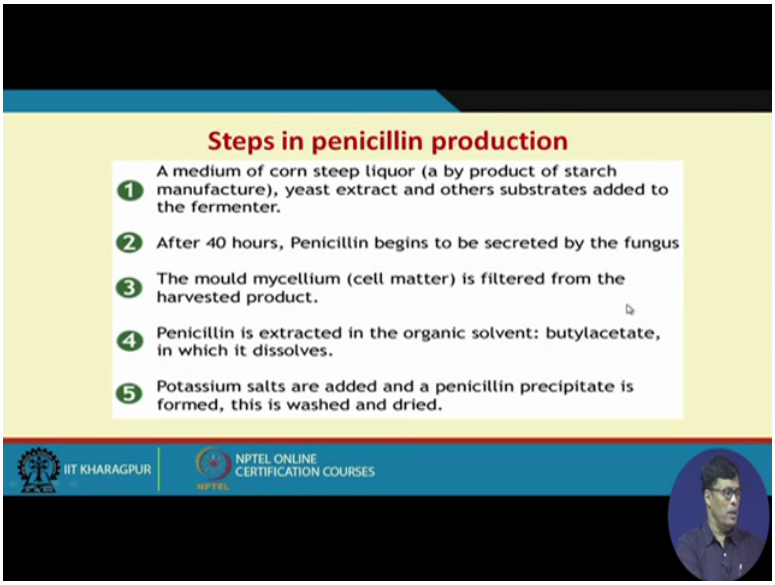

This is the steps involved in the penicillin production, a media is corn steep liquor in the by-product of the starch manufacturing industry, then corn steep liquor that we have and it is considered as a good nitrogen source, then we have yeast extract this also considered a good nitrogen source and other substrate added to the. I mention you before that, two type of media that we have, we have complex media, we have synthetic media.

Complex media means when the composition of the media is not well-defined but whenever you use any kind of natural product corn steep liquor is a kind of natural product that is produced from corn. So this and yeast extract also comes out from the yeast and other substance added other at particularly these two substrates if we present any of these 2 substance present in the media we can consider the media as a complex media, because the composition can be cannot be defined properly.

And after 40 hours penicillin begins to be secreted in the fungus. So the mould mycelia is filtered from the harvested product and the penicillin is extracted from the organic solvent butylacetate or amylacetate in which it dissolves and potassium salt are added in penicillin precipitate to form that is washed and dried.

Let me tell you the things how really it happens. So in the penicillin production because after the fermentation is over fermentation takes about 5 to 6 days after the fermentation is over we take out the fermentation broth and we separate out the cell by using rotary vacuum filter, then filtrate we take it out filtrate contains the penicillin, so penicillin is recovered by using the solvent extraction process, and solvent we use the butylacetate.

The butylacetate this now this is very critical process because in the lab you have Buchner funnel, with the help of Buchner funnel you have 2 different type of liquid solvent we use we can save it properly so that with as a solubility at the partition coefficient of particular solute is different in different layer different layer of solvent, so it move from one player to another layer, but industry we are handling huge amount of liquid, question comes how one how the penicillin present in the aqueous layer it goes to the sorbit layer.

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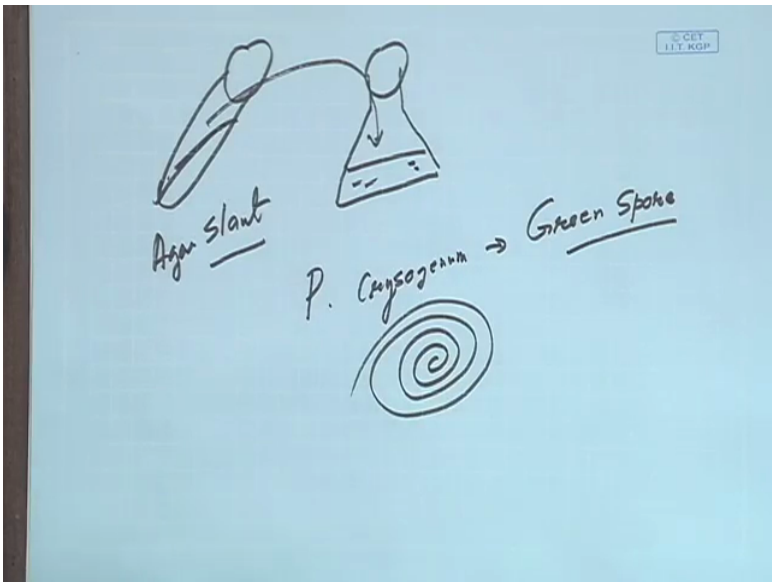

So they mix in a they have some kind of mixing like this they have through mixing they move they mix in such a way this both the layer can mix thoroughly and so that one layer that one that solid present in one layer goes to the other layer. But solubility depends upon the pH of the media we found that the acidic pH the solubility of penicillin is more in the solvent layer and in the normal in the neutral pH solubility of the penicillin little more in the aqueous layer.

So we know when we use the acidic pH then your penicillin will go to the solvent layer then this 2 butyl acetate is insoluble in water so you can easily separate the aqueous layer from the solvent layer and aqueous layer what you consider as industrial effluent we have to discreet it. Again you use some water and use some kind of buffer solution or so that you can maintain pH as 7, so that when you again mix together your penicillin will come back from the solvent layer to the aqueous layer. So then again you take this solvent out and again purify the solvent again you increase the decrease the pH to 2 by the help of acid then again you recovered solvent in the penicillin in the solvent layer.

Like this you can purify, because penicillin is to be purified properly because penicillin as I mention it is usually marketed into different forms either in the form of capsule or in the form of injection fluid. If it is the injection fluid, fluid is 100 percent free from the contaminants. So your fluid should not have any kind of contaminants present, because as soon as you inject your blast steam if any contaminants is there your health immediately will be affected.

So there is no contaminations should be 100 percent sterility you have to maintain. But in case of capsule that 100 percent sterility may not be required, because you know because as I told you that when you take in the form of capsule, it goes to the stomach, while pH is 2, if some microbial infection is there then your system will take into account. Then finally you use some kind of potassium salt, potassium salt might be potassium acetate or potassium carbonate we add so that potassium or potassium or sodium salt of penicillin can be separated out. And finally we do the crystallisation process, through the crystallisation process the penicillin salt or sodium salt we take it out from the system.

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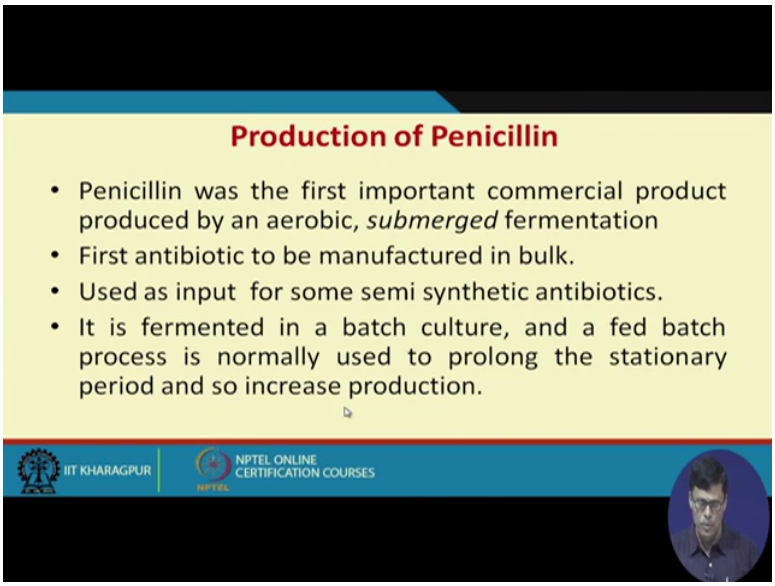

Now the penicillin was first the first important commercial product produced by aerobic submerged fermentation process. First antibiotic to be manufactured in bulk and used as input of for some semi-synthetic antibiotics I mentioned before. It is fermented in a batch culture, a fed batch process is normally used to prolong the stationary phase so increases to increase the production. Here let me tell you that particularly when the penicillin fermentation process, through the fermentation process we produce the Beta-Lactam group that this produce but actual production of penicillin depends on the precursor. We have seen the R group that is different for that very the different type of penicillin.

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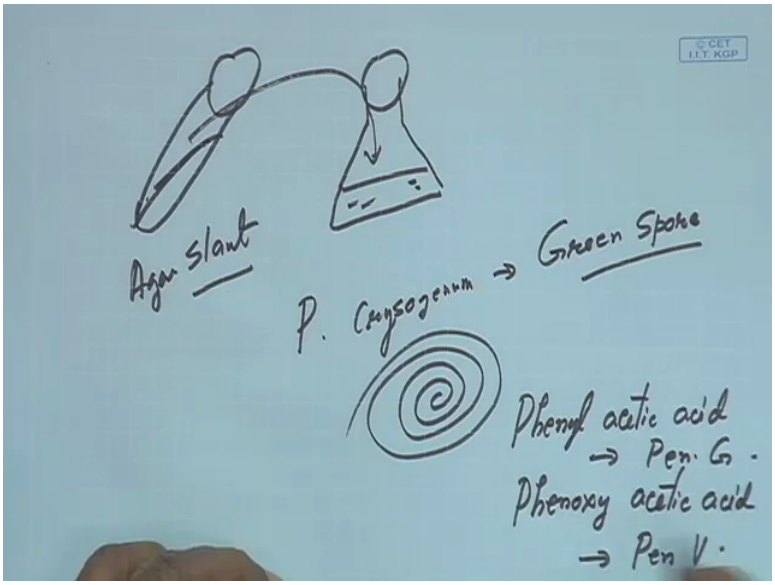

So we have in the fermentation process we usually produced two type of penicillin, one is penicillin-G and another is penicillin V. For the production of penicillin-G we use the phenyl acetic acid, phenyl acetic acid and this is in case of penicillin-G and in case of penicillin-V phenoxy acetic acid, penicillin V. So we use this 2 different precursor, this is the precursor that is required for the penicillin fermentation process.

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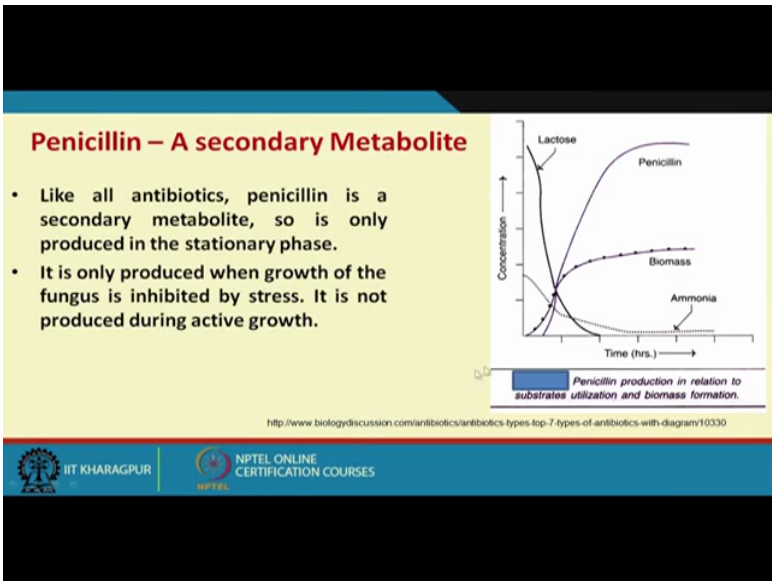

So this is the profile of the different component present in the reaction mixture. The lactose that is used as the carbon source this keep on decreasing respect to time and you can see that biomass production is increases like this and penicillin production is increases like this and ammonia that considered as a source of nitrogen they decreases like this.

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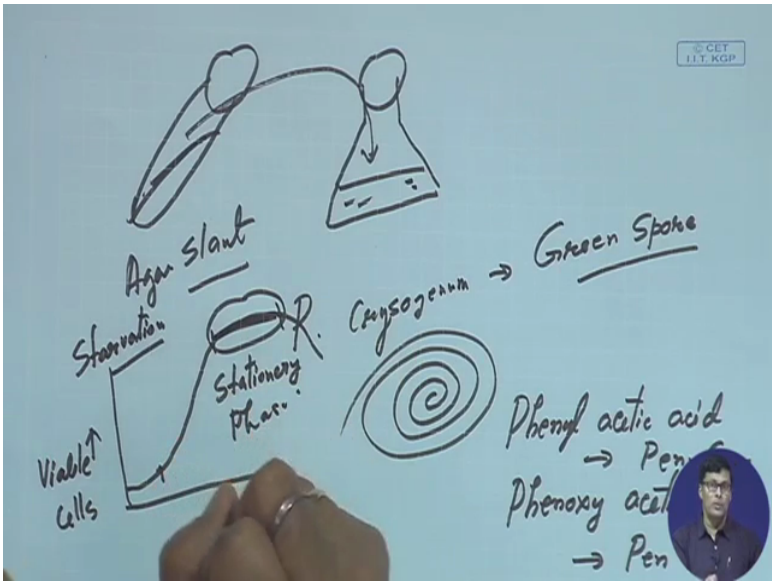

The like all antibiotics penicillin is the secondary metabolites, what is the secondary metabolite? I told you that when organism we have seen that life cycle of organism that this is the viable cells, this is time, so when you plot that is like this. So we have lag phase, we have log phase, we have stationary phase, we have depth phase. So this is called the stationary phase, now this stationary phase that what you have that we have this is called as the starvation phase this is called starvation phase.

So starvation means they do not have we do not have the sufficient substrate for the organism, so organisms struggle for survival that is the difference we have and like all antibiotics and during this process their metabolism little bit changes and so penicillin it produce there that is why we call it as a secondary metabolite. So it is only produced in the stationary phase. It is only produced when growth of the fungus is inhibited by stress and it is not produced during the active growth phase.

What you called here you look at this is considered as active board phase but here when you see that when the organism almost going to the stationary phase then your penicillin production started like this. So that is why we called it penicillin is considered as a non-growth associated product, because the rate of production is proportional to the cell mass but this will occurs only when the organism attends the stationary phase. Thank you very much.