Lec 14: Fermentation Industry – Citric Acid and Penicillin.

Welcome to the MOOCs course organic chemical technology. The title of today's lecture is Fermentation Industry, Citric Acid and Penicillin.

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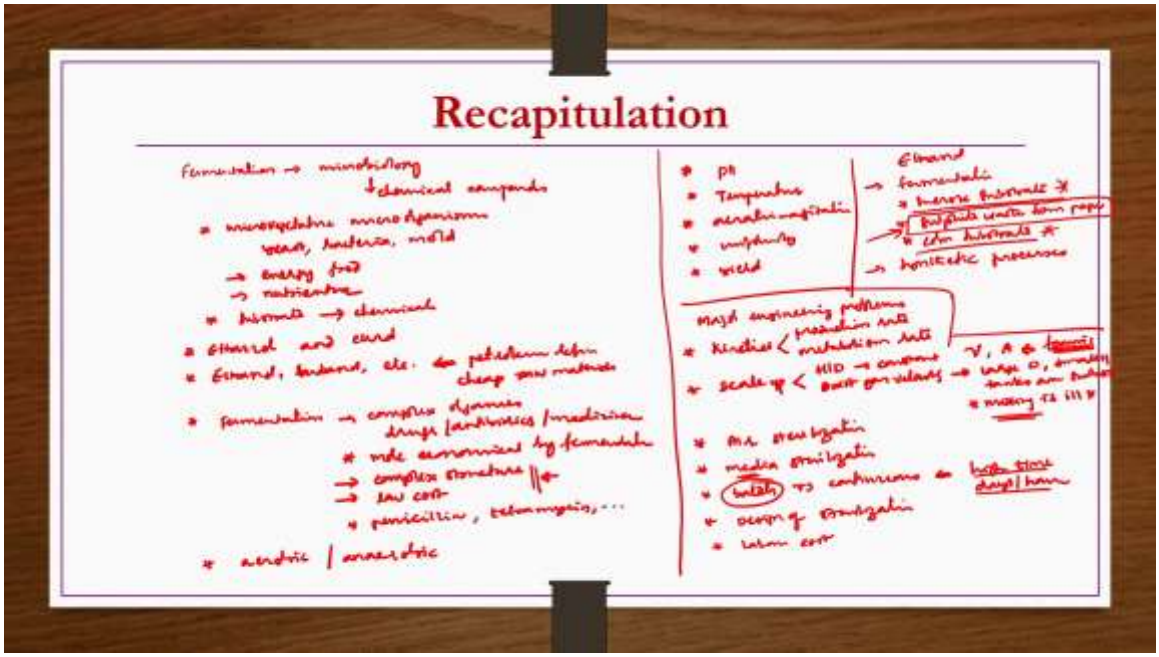

Before going into the details of today's lecture, we will have a recapitulation of what we have discussed in last couple of lecture on fermentation industry especially. Fermentation industry we started with the basics of fermentation and then we realized that it utilizes microbiology to produce chemical compounds. In this process it utilizes, it includes micro vegetative, microorganisms such as yeast, bacteria, mold, etc. And then when these are fed with energy food along with nutrients etc. if required.

Then what happen these will utilize the substrate or raw material, grow their size and then also produce some chemicals. Anciently conventionally this fermentation is well known for ethanol production, ethanol production and then cod production. But however, some simple chemicals like ethanol, butanol etc. are being superseded by so called you know petroleum refineries etc. because of the cheap raw materials and then process etc. So, because of such reasons nowadays ethanol, butanol etc. are not being produced by fermentation industry very much.

Though they are produced but their quantities, their percentage in overall ethanol production may be butanol production may be very less. However, fermentation is still used for some complex organics, something like drugs, antibiotics, drugs, medicines, etc. These are the more economical production by fermentation rather than by synthetic process

because complex structure, the structure is so complex that it is very difficult to reproduce or produce by synthetic methods and also by the low cost. Or the cost of production of the same complex structure even if they can be reproduced by synthetic methods, the cost of reproduction by synthetic methods is very high. So low cost and then complex structure by fermentation industry, these are primarily produced by fermentation something like you know penicillin, tetramycin, etc.

These kind of drugs, antibiotics mostly produced by the fermentation industry. Now coming to the generalized details of fermentation industry, we realize that they can be aerobic if they are utilizing oxygen or occurring in the presence of oxygen or air or anaerobic if they are occurring in the absence of oxygen or air. So, then we subsequently also we have seen, we have seen critical parameters that may be affecting the fermentation process. Those things are nothing but pH, temperature and then aeration, agitation, etc. and then uniformity, yield etc. are you know very important factors deciding whether the process of fermentation process is going to be economically feasible or not. Then most common engineering problems associated with almost all fermentation industries, those things are also we have listed out. So, in which we have seen the kinetics, right are very important. So, where we discussed about the production rate as well as the metabolism rate. Because the growth of yeast bacteria whatever we have taken, they have to be specific towards the product and then substrate should also be specific towards the product.

So, when the combination meets properly, then their growth is also important, growth of the yeast bacteria, mold, etc. their growth is also important. So, their metabolism rate is also very essential. Then scale up issue, right h by D if you keep constant and then you try to change V or A exit velocity, etc. So, then there are going to be forming issues, too much of forming may take place, right.

So now what you do? You keep exit velocity, exit gas velocity because gases would also be forming, if that you keep constant and then if you take H and D variation, large D, small h tanks are suitable or the tanks with large diameter and then small height are suitable if you take the scaling parameter by exit gas velocity as a constant one. Then but here the problem is that mixing is not proper. So here the forming problem is there, here the mixing problem is there. So that is the reason scaling is a kind of a compromise amongst the different type of parameters they are involved. Then any of the fermentation, sterilization is very essential, right.

Because we understand that fermentation industry, you know impurities are very dangerous, even little bit impurities, the micro vegetative microorganism may be destroyed or it may not be producing the required products or it may be producing unnecessary products etc. those kind of issues may there. Then also media sterilization, whichever media substrate you are taking by on which you are you know releasing these yeast bacteria, etc. microorganisms to produce chemicals so that media has to be sterilized

properly. Otherwise that may also lead to some kind of impurities or unnecessary products, which may subsequently increase the both capital cost as well as the operational cost in terms of separation requirements, right. Then batch versus continuous process also we have seen.

In such cases like fermentation where especially high residence time or high process time is there in fermentation hours or sometimes days also there. If you are able to do in a few hours, it is really rapid. If you are able to complete the fermentation in minutes, it is very, very rapid. So, because of high residence time or process time, most of the fermentation processes are batch process though the separation processes like you know subsequent separation or purification by distillation etc. they would be done continuously but fermentation is batch process only, right because of such reasons.

Then design of sterilization operations are very essential because sterilization is very essential. It is not only the air and then media etc. but it is required even for the so called you know this entire equipment, joints etc. all of them properly sterilized. So, design principles of sterilization are also important and then labor cost also very essential.

So, this is what we have discussed and then what we discussed about the production of different types of chemicals and then we started with ethanol production, fermentation processes there and then synthetic processes also there or petroleum refinery approaches is also there. Under the fermentation processes what we have then sugar sucrose or sugar substrate we use and then sulphite waste from paper and pulp industry and then you know corn substrate we can use and then produce. Out of these three, two processes we have discussed in the previous lecture, the sulphite waste from pulp and paper method, this probably we discuss in the next week where we will be discussing about the pulp and paper industries. So, this is what we have discussed in last couple of lectures on fermentation industries. Now we are going to discuss about production of different chemicals.

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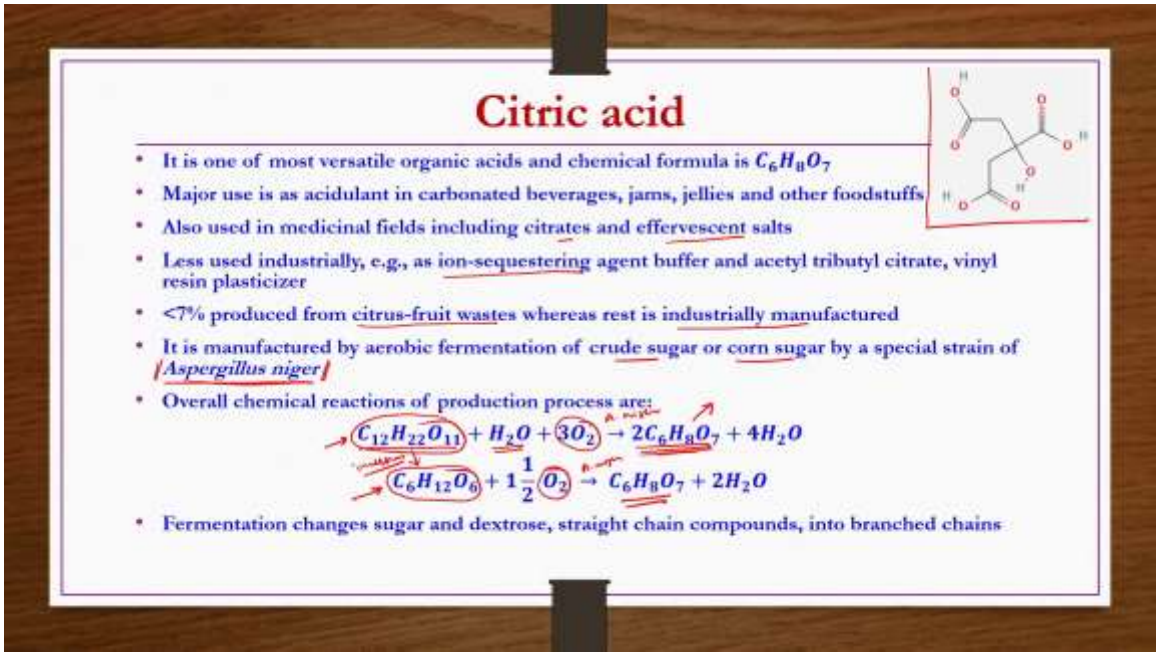

We start with citric acid. Citric acid it is one of the most versatile organic acids and chemical formula is $C_6H_8O_7$ it is a branched organic chemical and the structure is shown here it is a branched structure here as shown here. Major use is as acidulant in carbonated beverages, jams, jellies and other foodstuffs, etc. It is also used in medicinal fields including citrates, effervasins, salts, etc. for those purpose used.

However, in industries it is used very less example for ion sequestering, agent buffer and then acetyl tributyl citrate and vinyl resin plasticizer for this purpose it is used industrially. And then coming to the overall production citric acid production only 7 percent is produced from this citrus fruit waste whereas, rest is industrially manufactured. It is manufactured by aerobic fermentation of crude sugar or corn sugar by a special strain of Aspergillus niger. Aspergillus niger this is what we are going to use as a microorganism or micro vegetative organism and then we feed crude sugar or corn sugar along with some nutrients to these microorganisms then the strain will grow and then they will convert these sugars, etc. into citric acid. How that is going to be occurring that we are going to see subsequently in flow chart anyway. Overall reactions of production process if you see whatever the sugar or sucrose is there that is nothing but $C_{12}H_{22}O_{11}$ if it reacts with water in the presence of oxygen or air, then what happens it gives citric acid it does not give easily what you have to do you have to use this Aspergillus niger micro vegetative microorganism then this will be consumed by this microorganism in the presence of oxygen and water and then acetic acid would be produced. This acetic acid production once it is done purification unit operation should be there and then appropriately we purify it. How we see the flow chart anyway. Other reaction possible this sucrose or sugar whatever is there that may undergo

some kind of inversion reaction as we have already seen in the previous chapter on sugar and starch industry.

When the sucrose undergo inversion, inversion is nothing but you know the sucrose when it hydrated during the early stage of sugar production from the sugar cane, then you know D-glucose or D-fructose they form that is nothing but $C_6H_{12}O_6$ and then that one if you oxidize using oxygen in the presence of the same Aspergillus niger micro vegetative microorganism then also you can get the citric acid. Fermentation changes sugar and the dextrose which are straight chain actually these are straight chain components that we have already seen in the sugar industries, whereas the citric acid that is produced that is a branched one as shown here. See it is a branched structure.

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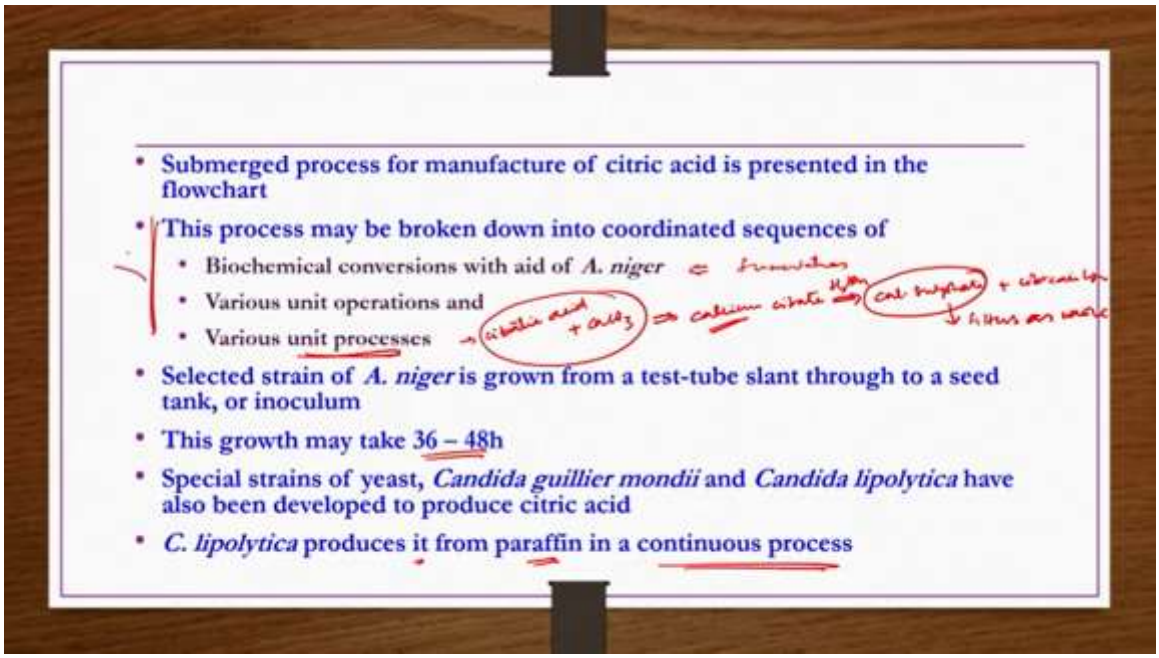

Submerged process for manufacture of citric acid is presented in the flow chart. This process may be broken down into coordinated sequence of obviously biochemical conversion with aid of Aspergillus niger, A niger.

So, then this one it is nothing but the fermentation reaction. In this fermentation process what happens citric acid forms but it is not in the pure form. So, it has to be purified for that purpose some unit operations like filtration process, etc. Then ion exchange processes and then packed bed processes, drying processes, etc. are required. So, those are there and also some unit processes are also there because in the process of purification you might be reacting the citric acid or the solution that is containing citric acid after the fermentation. So, that you might be reacting with you know calcium carbonate. So, in this process calcium citrates may form. So, this calcium citrates you may be reacting with H_2SO_4 to

remove the calcium part in the form of calcium sulphate plus citric acid liquid or liquor. So, this calcium sulphate again you separate out using the filter press as waste.

So, such kind of reactions are involved here. So, because of that one there maybe we are listing some other unit processes as well along with the fermentation biological conversion process. Selected strain of Aspergillus niger is grown from a test tube slant through to a seed tank or inoculum. This growth may take 36 to 48 hours. Special strains of yeast Candida, Guilliar, Maundy and then Candida lipolytic have also been developed to produce citric acid.

Whereas Candida lipolytica produces citric acid from paraffin in a continuous process.

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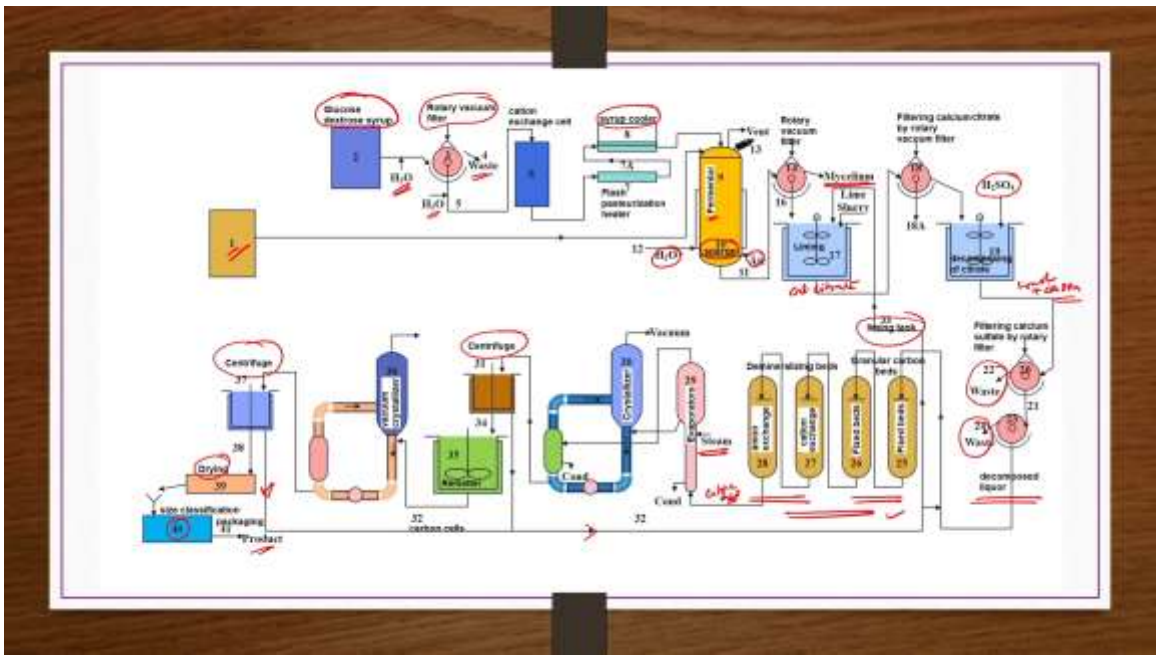

However, we are going to discuss about this process only in the form of flowchart as shown here. So, what happens here in the plant where you are producing citric acid by fermentation process using glucose dextrose syrup that whatever the glucose dextrose syrup is there that you dilute, you dilute it. When you dilute it what happens some kind of precipitates or solid impurities whatever are there they can be easily removed. If you do the dilution that is one advantage of dilution.

Another advantage of dilution if the substrate is diluted then inoculation may go effectively by using the microorganism vegetative that is other point. So, solid impurities, etc. are removed by passing this dilute solution through rotary vacuum filter. So, solids are taken as waste then whatever the filtrate is there you further dilute it then pass through cation exchange cell. If the trace elements impurities, etc. are there in order to remove that this

cation exchange cell would be useful. Then after that you pass through a flash pasteurizer heater to further you know do the required sterilization of the substrate and then it will pass through a heater and then syrup would be cooled in syrup cooler. Because the temperature here when you do the sterilization may increase but you know at higher temperature you cannot do fermentation. So, before sending the dilute syrup to the fermenter it has to be cooled. So, whatever the syrup solution is there that would be sent to the fermenter to which what you do you can send so called you know aspergillus niger micro vegetative microorganism after sterilization.

Then here in the fermenter required fermentation reaction takes place but this reaction would be aided by air. So, air is passed from the bottom of the reactor to the fermenter continuously. So, while this reaction is going on there may be possibility of that you know heat increasing. So, then cooling is supplied for in order to maintain the temperature less than 30 to 32 degrees centigrade.

If any vapors gases etc. are formed they will be taken out as vent from the top whereas the liquid beer whatever is there that would be containing the citric acid. Now, that citric acid may also be containing some kind of undesired elements like mycelium they would be removed by rotary vacuum filters. After that whatever the liquor is there that would be limed using the lime slurry because of this one pH is balanced but however though you are adding this one to maintain the pH some liming action takes place because of that one what happens you know calcium citrates may form those calcium citrates you try to remove by the rotary vacuum filter further here in the form of precipitates etc. If it is not possible to remove there then what you do you react that one using the so-called H_2SO_4 so that here liquor you get citric acid liquor plus so-called calcium sulphate you can get. This calcium sulphate can be easily washed out and then removed by using rotary filters as shown here.

So, whatever the calcium sulphate is that is collected as waste from here. So, decomposed liquor in the sense it is whatever the citric acid is liquor only but after sulphuric acid decomposition reaction is coming so that is the reason it is known as the decomposed liquor. This liquor is further sent to fixed beds where granular carbon is used as a packing medium then they will also pass it through demineralizing beds something like ion exchange reagents or cation exchange reagents would be added so that to remove any trace impurities or minerals, etc. are there so those things would be removed by these 2 steps. Now here what you get almost you know clear citric acid you get without any impurities but however it may be dilute so then what you do you increase its concentration by the multiple effective operators by using steam.

So, when you heat it using the steam so citric concentration increases that concentrated citric acid would be taken to a crystallizer where vacuum is applied so that vacuum crystallization takes place. Whatever the crystals that you get from here the crystals may also be containing some amount of you know liquor or syrup etc. So, that liquor or syrup

etc. that you remove by using centrifugation process. Centrifugation process by now we have already seen in the previous lecture what is centrifugation how does it occur and all those things.

So, after centrifugation whatever the liquor is there that is collected and then sent back to the liming tanks for the liming purpose. Whereas the crystals that you get that you do the remelting and then you redo the vacuum crystallization if at all if any impurities are there they would be removed by subsequent centrifugation process. Then those crystals whatever are there after removing the syrup and then other traces of impurities if any the clear citric acid crystals whatever are there you do the drying whereas the solution or syrup whatever is there that is sent to carbon cells or the liming tanks. Carbon cells in the sense here it can be used here in this process granular carbon weights or they can be used in the liming tanks. So, after doing the drying of the crystals what you can do you can check the size classification you have to do if the crystal size is as per the requirement then you can go for the packaging and product otherwise you can send back them to the crystallizer and then try to improve the size of the crystals as per your requirement.

So, this is what fermentation process to get the citric acid from glucose dextrose syrup using Aspergillus niger micro vegetative microorganism.

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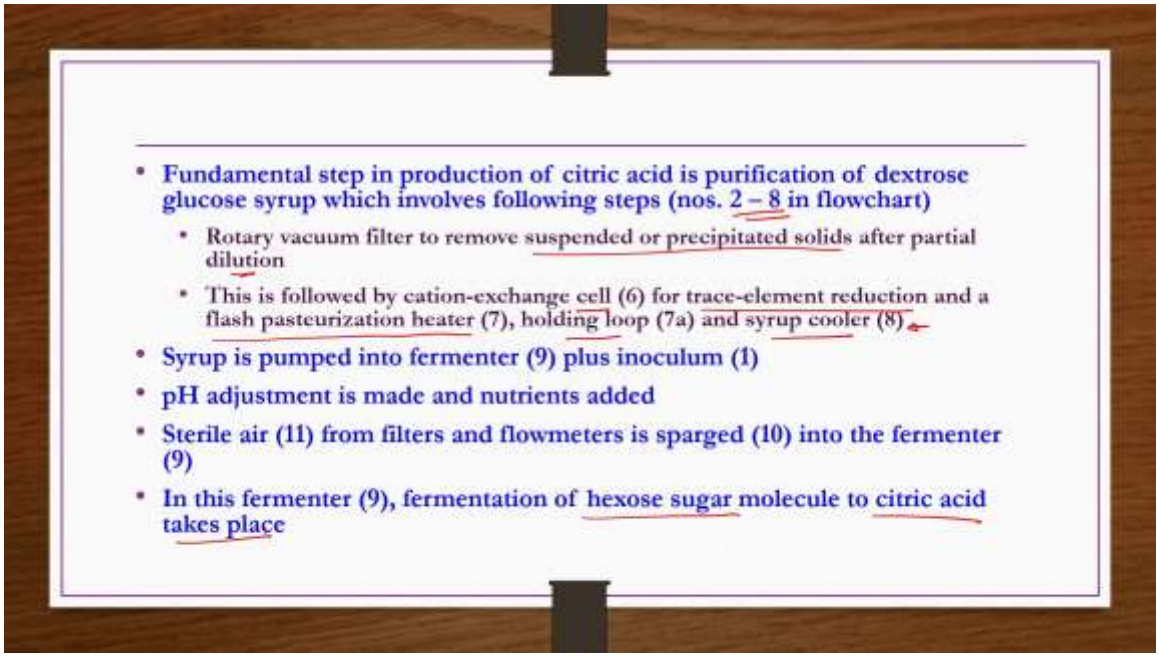

So, whatever description that we have discussed on the flowchart the same is provided here. These numbers are the numbers that are shown in the equipment part here like you know everyone and these numbers equipment or unit operation unit processes streams are given numbers. So, the same numbers have been used here in the text as well for

understanding point of view. Fundamental step in production of citric acid is purification of dextrose glucose syrup which involves following steps rotary vacuum filter to remove suspended or precipitated solids after partial dilution with water.

This is followed by cation exchange cell for trace element reduction and flash pasteurization heater holding loop and then syrup cooler. So, this is by this one sterilization is taken place and then cooling has also been taken place because in the sterilization temperature rises but at high temperature if you use the media required fermentation is not possible that is the reason cooling is done. Syrup is pumped into fermenter and plus inoculum so that the required fermentation takes place. pH adjustment is made and nutrients are added if required. Sterile air from filters and flow meters is passed into the fermenter because it is a aerobic process.

So, then air has to be sparsed from the bottom it is done. In this fermenter fermentation of hexose sugar molecule to citric acid takes place.

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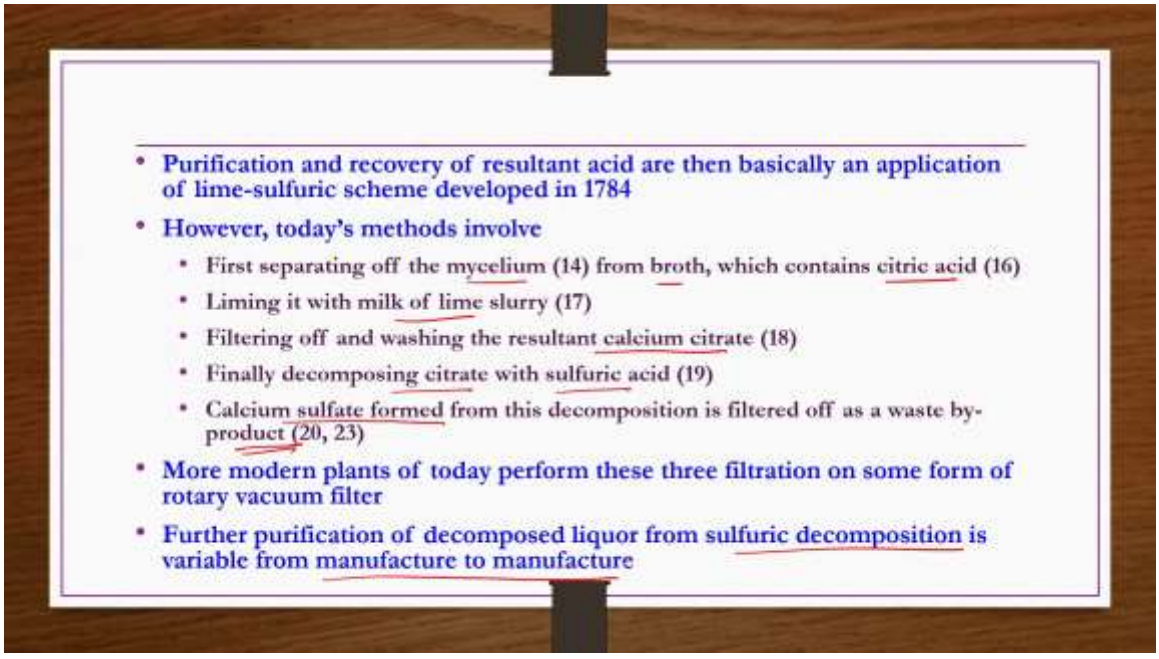

Purification and recovery of resultant acid are then basically an application of lime sulphuric scheme developed in 1784. However, today's methods involve following that is first separating of the mycelium from broth which contains citric acid, liming it with milk of lime slurry, filtering off and washing the resultant calcium citrate followed by finally decomposing calcium citrate with sulphuric acid and then calcium sulphate formed from this decomposition is filtered off as a waste by product. More modern plants of today perform these 3 filtration on some form of rotary vacuum temperature.

Further purification of decomposed liquor from sulphuric decomposition is variable from manufacture to manufacture.

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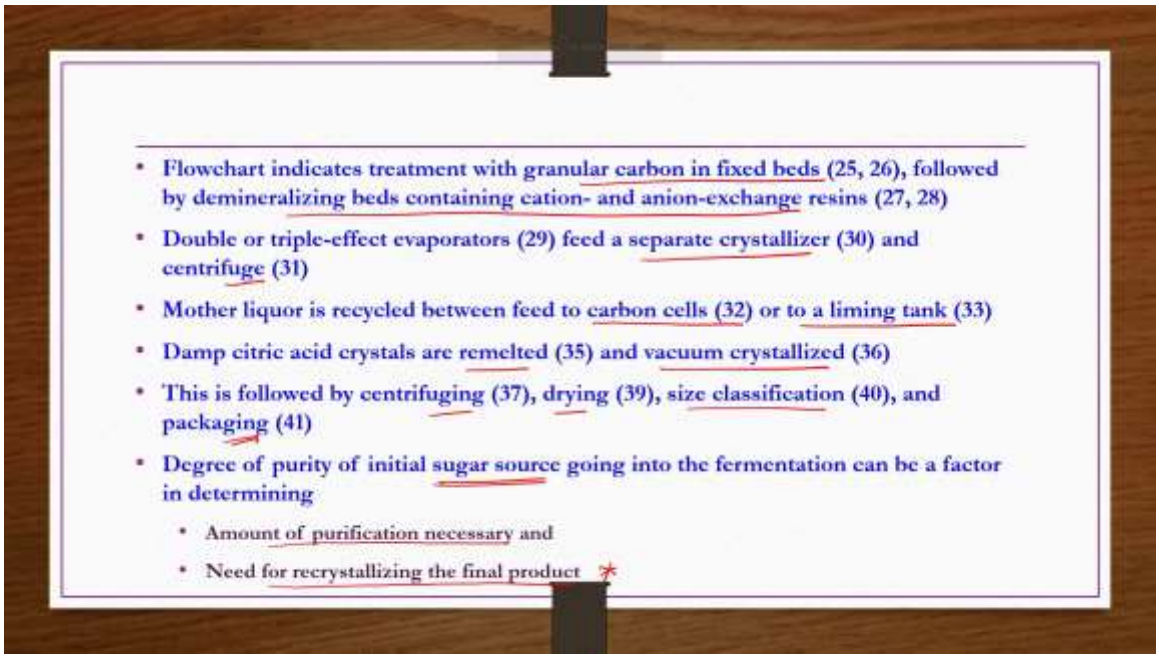

However, the method that we discussed is presented here. Flow chart indicates treatment with granular carbon in fixed beds followed by demineralizing beds containing cation anion exchange resins followed by once you do the demineralization of the citric acid liquid then double or triple effect evaporators feed a separate crystallizer and centrifuge for the concentrating of liquid and then forming the crystals. Mother liquor is recycled between carbon cells or to liming tank as shown in the flow chart. Damp citric acid crystals are remelted and vacuum crystallized.

This is followed by centrifuging to remove the if at all traces of syrup etcetera are there along with the crystals then drying then size classification and packaging. Degree of purity of initial sugar source going into the fermentation can be a factor in determining especially amount of purification necessary then need for recrystallizing in the final product. If you see in the flow chart we have done the recrystallization 2 times. So, it may not be required if you have the initial sugar source as pure enough. If it is not pure enough then sometimes you know second time or recrystallization after remelting recrystallization may also be required if the initial sugar is not very pure enough.

If it is very pure enough then you can do the crystallization in one step only you do not need to do the second step crystallization.

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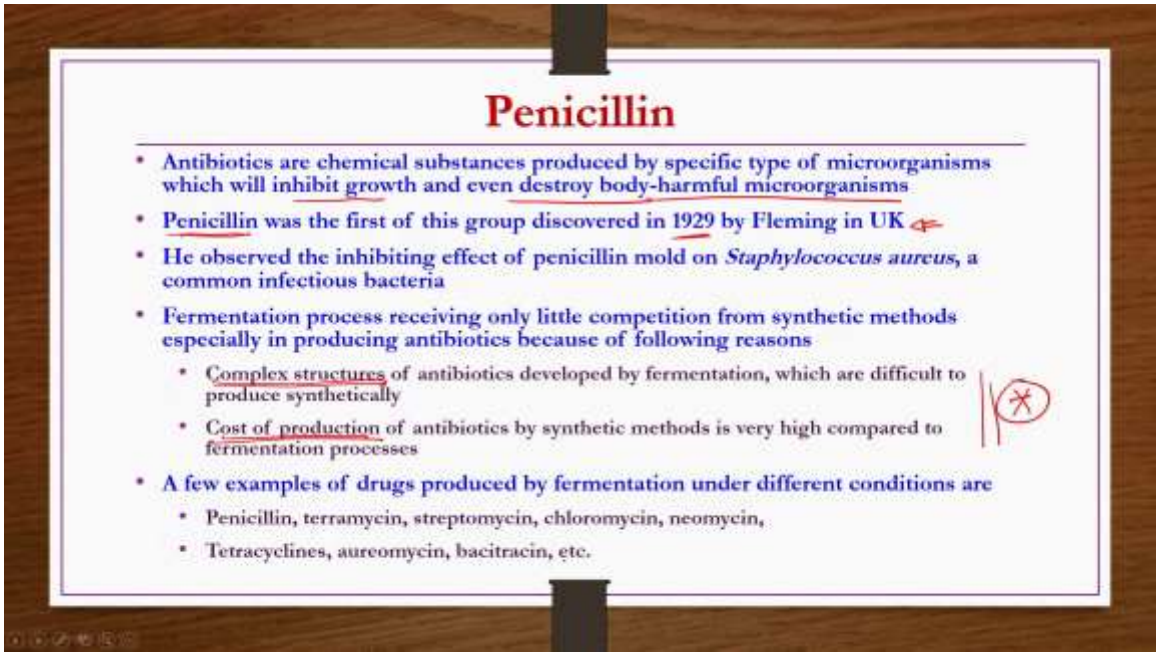

Now we discuss about antibiotic penicillin how it is produced by fermentation process. Antibiotics are chemical substances produced by specific type of microorganisms which will inhibit growth and even destroy body harmful microorganisms. Actually, these are also whatever the antibiotics that are we are developing they are one or other kind of chemical productions because of the fermentation. Actually, what happens this antibiotics when you take so whatever the body harmful microorganism bacteria etcetera are there their growth would be inhibited by these antibiotics.

Even sometimes these antibiotics kill such you know body harming microorganism that we already know. Penicillin was the first of this group discovered in 1925 by Fleming in UK during the Second World War and then because of this development you know the fermentation industry has taken boom again and then it is started competing with the synthetic processes as well. Otherwise that time fermentation industries were found to be uneconomical because synthetic petroleum refinery process were able to produce so called ethanol, butanol at much lower cost compared to the fermentation and then quickly as well. But the production of penicillin by fermentation has given a new direction to the fermentation industry and then after that fermentation industry has not looked back and then it was progressing forward only in developing of several types of antibiotics drugs which cannot be developed synthetically in economic manner compared to fermentation process. He observed the inhibiting effect of penicillin mold on Staphylococcus auris a common infectious bacteria.

Fermentation process receiving only little competition from synthetic methods especially in producing antibiotics because of two reasons. One is the complex structures. These

complex structures are very difficult to develop by synthetic methods. So, this whatever the antibiotics developed by the fermentation process they are having complex structures and then they cannot be easily produced by synthetic methods. First of all that is one reason even if they can be produced synthetically the cost of production by synthetic method is much very high compared to the cost of production by fermentation process.

So, because of these two reasons, you know fermentation industry is really dominating in the area of antibiotics and then different types of drugs. A few examples of drugs produced by fermentation under different conditions are penicillin, teramycin, streptomycin, chloromycin, neomycin, tetracyclines, oryomycin, bacitracin, etc.

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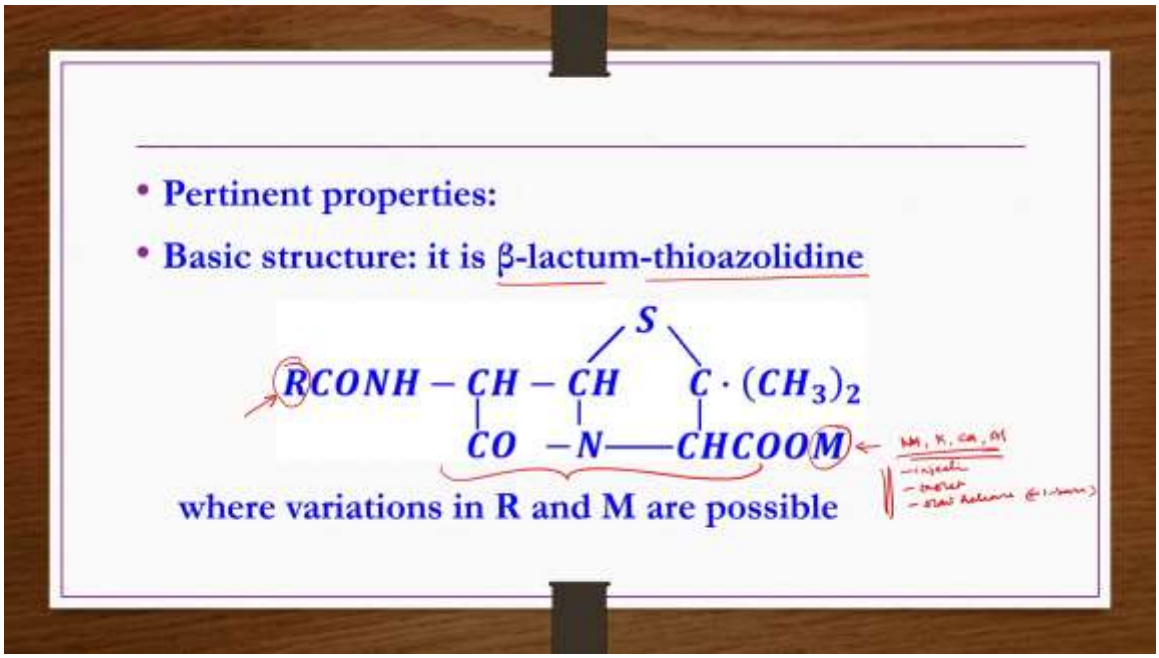

Now, we see pertinent properties of penicillin after having a little background on antibiotics by fermentation industries. Basic structure it is given it is beta-lactam thioazolidine. So, here two rings are there one is the side ring and then this is the main ring.

In the main ring you have this M. So, this M is usually sodium, potassium and then calcium, alumina, etc. this kind of minerals depends on how what form of application it is like injection, quick delivery of medicine to the body that injection or by tablet or by slow release injection by oil base. Depending on these requirements of how you wanted to use this penicillin, this M would not be changing how it is that is we are going to see and then depending on what type of this side chain R is there.

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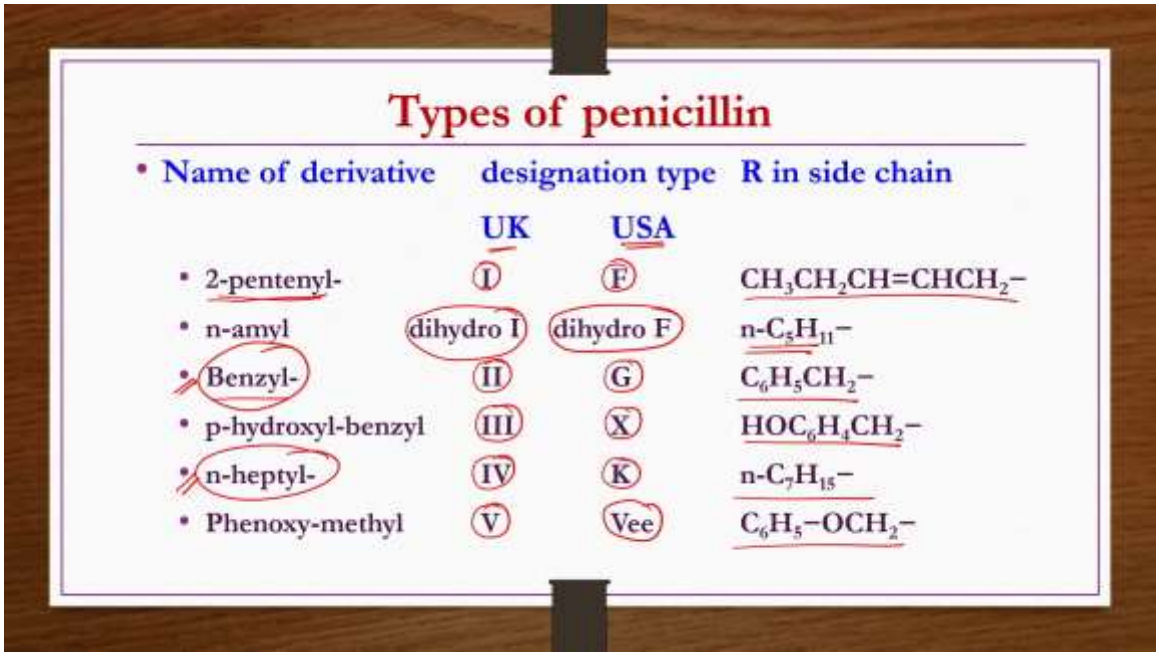

So, different types of penicillins are possible those things we are going to see now. Let us say if your R in side chain is having this structure CH_3 CH_2 CH CH CH_2 then it is known as pentenyl derivative actually in UK and US different designations are there in UK it is known as the penicillin 1 whereas in USA penicillin F.

Likewise, n-amyl derivative then R in side chain you will be having $n\text{-}C_5H_{11}$ in UK it is known as dihydro 1 and then in USA it is known as dihydro F. If you have benzyl in side chain like $C_6H_5CH_2$ that is known as the benzyl derivative UK it is known as penicillin type 2 and USA it is G. Likewise P hydroxyl benzyl then it is type 3 in UK and then type X in USA. Structure of R in side chain is this one. If it is n-heptyl then in UK it is known as penicillin 4 type in USA it is known as penicillin K type and then structure of R is this one $n\text{-}C_7H_{15}$.

Finally, if you have phenoxy methyl then in UK it is known as penicillin 5 USA penicillin V and then this is the structure of R in the side chain. Coming to the medicinal application point of view these 2 benzyl and then n-heptyl derivatives are found to be having a lot of market utilization or applications in medicinal fields.

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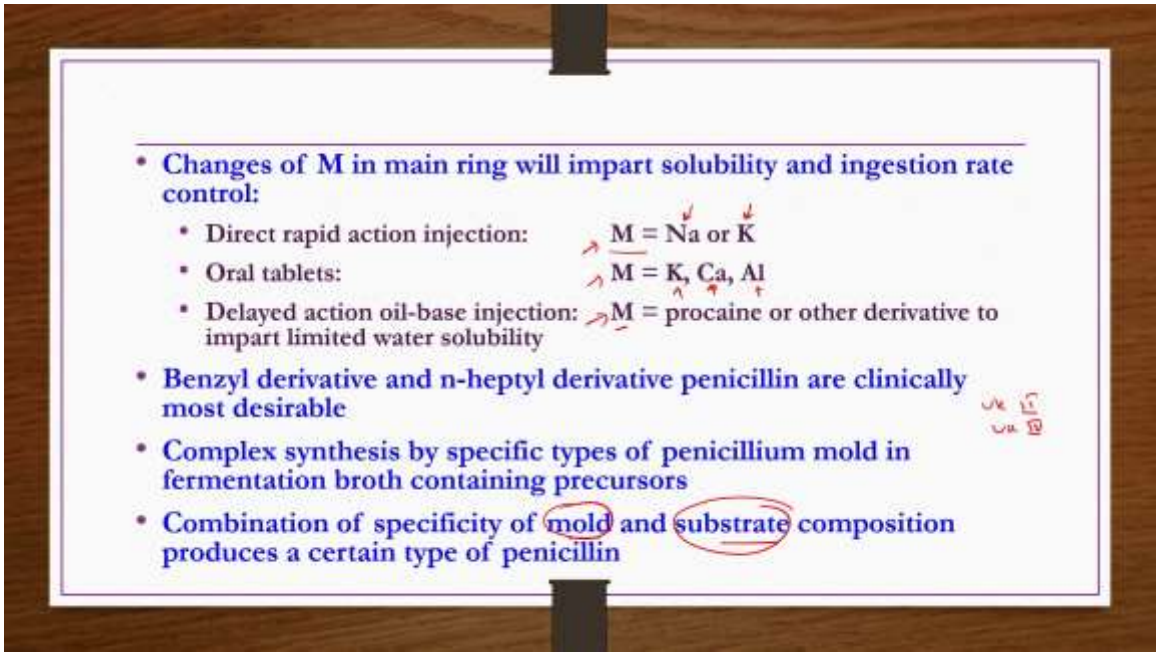

Changes of M in main thing will impact solubility and ingestion rate as well. So, direct rapid action injection if you required then M has to be either sodium or potassium.

Oral tablets then M has to be K potassium, Ca calcium or aluminum Al. Delayed action oil-based injection if you use as application method then M has to be procain or other derivative to impart limited water solubility. Benzoin derivative and n-heptyl derivative penicillin are clinically most desirable that is UK type 2 and then UK type 4 are more desirable clinically. Complex synthesis by specific types of penicillin mold in fermentation broth containing precursors. Combination of specificity of mold and substrate composition produces a certain type of penicillin. Actually 5 types are there so then accordingly substrate and then mold you have to decide accordingly you have to have the other type of these M's etc. all those things.

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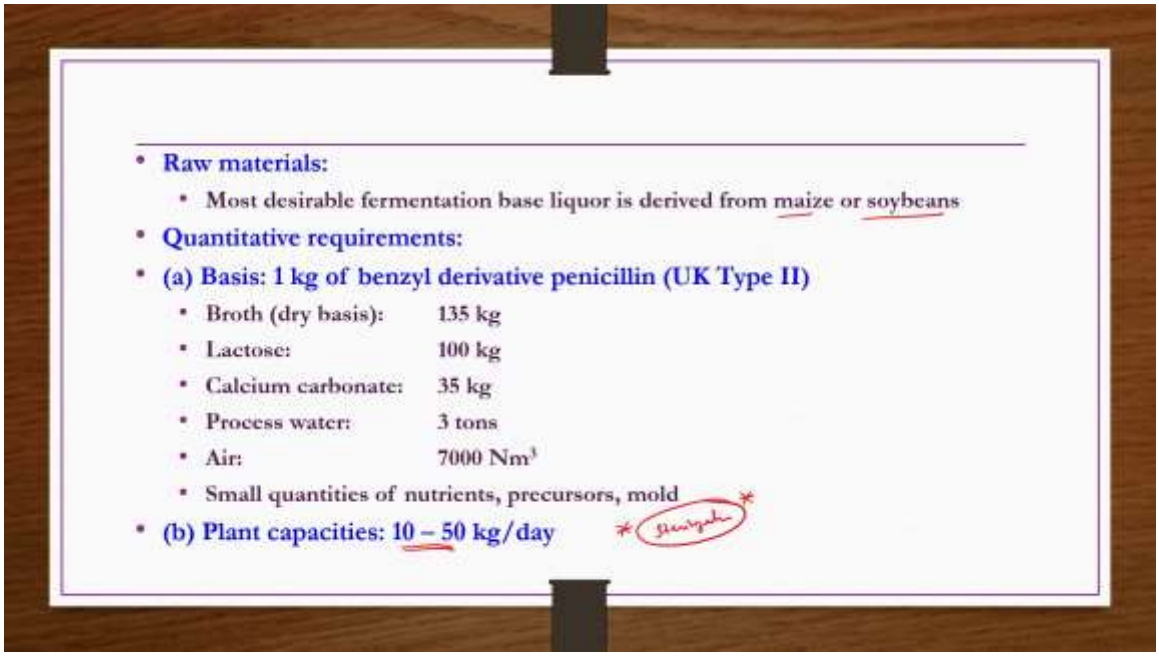

How that is what we are going to see anyway. So, let us start with the raw materials. If you wanted to produce type 2 UK type 2 then most desirable fermentation-based liquor is derived from maize or soya beans for the penicillin production. It is for the type 2 UK type 2 penicillin. Quantitative requirements 1 kg of benzyl derivative penicillin that is UK type 2 if you want to produce. Then broth on dry basis 135 kg required, lactose 100 kg required, calcium carbonate 35 kg, process water 3 tons, air 7000 normal cubic meters, small quantities of nutrients, precursors, molds, etc. required and then plant capacity is not much 10 to 50 kg per day because lot of sterilization is required.

It is an additional application. So, then you have to be very clear about the purification level standard of the purity, etc. So, that is the reason it is prepared in the small quantities and then carefully it is prepared.

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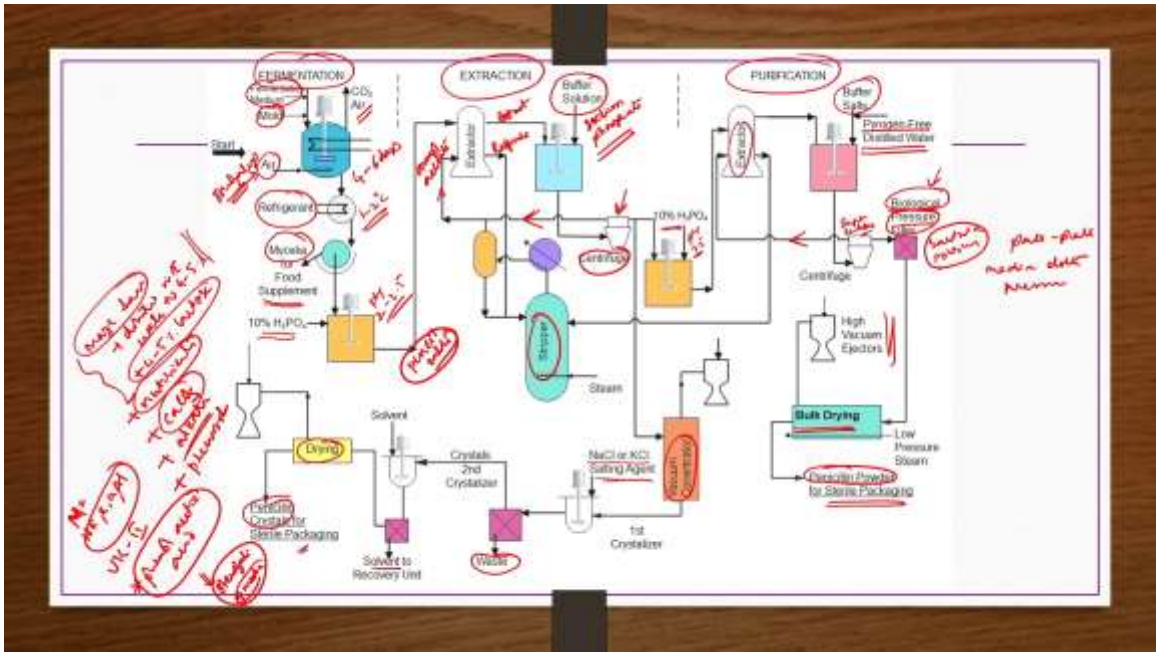

Now, here flowchart for the production of penicillin is shown penicillin in the powder form penicillin in the crystalline form as well. So, here what we have we have a fermenter to which air is being sparse from the bottom because it is also forming by aerobic fermentation process, whatever the fermentation medium mold, etc. they are added to the fermenter reactor and then air is being supplied from the bottom.

So, then fermentation takes place 4 to 6 days. It is not in few hours. It is 4 to 6 days in general takes to complete the fermentation process. Once the fermentation process is there then subsequent extraction purification is there. So, basically this flowchart you can see as 3 stages fermentation stage followed by the extraction stage followed by the purification stage. So, we take one by one. So, before going into the fermentation what you have to do this fermentation whatever is there maize, steep liquor or similar base whatever is there that is diluted with water.

So, that 4 to 5 percent solids would be there and then it is also added with 4 to 5 percent of lactose and then thoroughly mixed it. After that if at all required nutrients, nutrients should also be added. All these things are before dumping and this media whatever the fermentation media is there before putting into the fermentation reactor all these things are required to be done. Then also you have to do calcium carbonate addition to maintain the pH between 5 to 6. Then some kind of nutrients alkali etcetera to be added whether in the final form M has to be sodium or potassium calcium or aluminum based on that requirement alkalis has to be added here.

Then also precursors also because now you are doing this UK type 2 penicillin production so then precursor has to be like you know phenyl acetic acid precursor. So, depending on the which type you are making so accordingly this precursor is required depending on what type of alkali metal is required in your product. So, then accordingly alkaline salts has to be added then calcium carbonate has to be added to maintain the pH then nutrients should be added lactose should be added and then should be diluted with water so that to make that 4 to 5 percent solids are there. So, this all has to be done to the made steep liquor or similar base whatever you are taking as a fermentation medium. After doing all these things what you have to do you have to do the sterilization of a media this is very essential.

Then this sterilized media you have to provide to the fermenter reactor and then this air has to be sterilized this air should also be sterilized. Sterilized air has to be passed into the reactor from the bottom. So, this fermentation medium along with the reactor and then mold whatever the type of pencil in you are trying to prepare accordingly the type of mold will change they will be taken to the reactor and then reaction takes place 4 to 6 days during the reaction some CO_2, etc. may be forming so those things are usually removed from the top continuously. Then after the reaction completed or fermentation process is completed penicillin salts might have formed. So, that salt may be having some impurities also let us say mycelia, etc. would be there so that you can separate and then you know use as a food supplement.

But that can be taken place only if you are doing the filtration at 1 to 2 degrees centigrade. So that is the reason before the filtration the product of the fermentation process whatever is there that should be passed through your refrigerator. So, that the temperature is 1 to 2 degrees centigrade. So, at that temperature if you do the rotary vacuum filtration then mycelia you can easily remove.

After removing the mycelia what you can do you can add 10 percent of phosphoric acid. So, why are we adding this phosphoric acid here to maintain the pH of 2 to 2.5 in order to maintain pH of 2 to 2.5 you are adding this phosphoric acid. So, after this process what you get you get penicillin salts but it is not pure enough.

So, it has to be properly purified. So, for that purpose extraction process is there how it is extracted using the amyl acetate solution. When you use the amyl acetate solution and then mix with this pencil in salts then whatever the raffinate or water phase etcetera is there that you get from the bottom that you take to the stripper section recover the solvent once the recovery is done that solvent is fed back. Whereas the extract is there that would be having you know primarily penicillin salt base that would be further added to a mixture where the buffer solution sodium phosphate is added. So, this is required in order to maintain the so-called required sodium requirement in the penicillin etcetera. So, once you do the buffering using the sodium phosphate whatever the solution is there that you take it to the centrifuge.

In this centrifuge you separate the liquor or solution solvent that solvent you whatever you recovered that you feed back to the extractor. So, whereas the clear salt whatever is there that you can do another stage of extraction or you can take to the other process of vacuum concentrator. So, let us say if you want to make more and more purified product the second acid extraction actually we are doing extraction using the phosphoric acid etcetera followed by the amyl acetate solution adding. So, then here after removing the solvent whatever the purified penicillin salts are there further you mix with the 10 percent phosphoric acid so that here pH is around 7.5. And now this solution again you take to the extractor extract the product penicillin salts and then here buffer salts to be added along with the pyrogen free distilled water for the purification. So now here this after this buffer salts addition and then pyrogen free distilled water addition that mixture is taken to super centrifuge that means it centrifuges at very high speeds more than 10,000 rpm etcetera 10,000 12,000 rpm etcetera such high speeds it has to be used. So, then here again whatever the liquids solvents you recover after centrifugation so that you can say fed back to the extractor whereas the salts you get that you pass through biological pressure filter to remove any bacteria if at all present or pyrogens etcetera have come into the salts so they will be removed by biological pressure filter. These pressure filter are same as you know so called plate and frame filters and then rotary vacuum filters those things we have seen so here plate and frame filter only. But we call them biological pressure filters because their medium filter media or the cloth whatever is there filter medium cloth etcetera requirement applied pressure etcetera such a way that you know bacteria is removed.

After removing the bacteria whatever the pure penicillin is there penicillin derivative is there that would be dried using a vacuum dryer and then penicillin powder is collected for sterile packing here also sterilized before packing also it has to properly sterilized. Other option is that you know if you wanted to get the crystals after this process after this first centrifuge after this first acid extraction whatever the penicillin salt is there that you take to a vacuum concentrator increase the concentration of the penicillin salt or you know penicillin derivative by applying the high vacuum. Then whatever penicillin derivative is there that you take to a mixture where you add NaCl or KCl salting agents as per the requirement. Then you do the filtration process to remove if at all any waste etcetera are there. Then again you do the crystallization by taking into another mixture followed by the solvent recovery and then once you have done removal of solvent and then impurities as much as possible as per the requirement rather as much as possible as per the requirements of the pharmacopoeia.

So, you have to do that one once the sufficient purity has arrived and what you can do you can do the drying. Drying, you can do the spray drying or vacuum drying and then once you dry the crystals of penicillin you get that should also be sterile before packaging.

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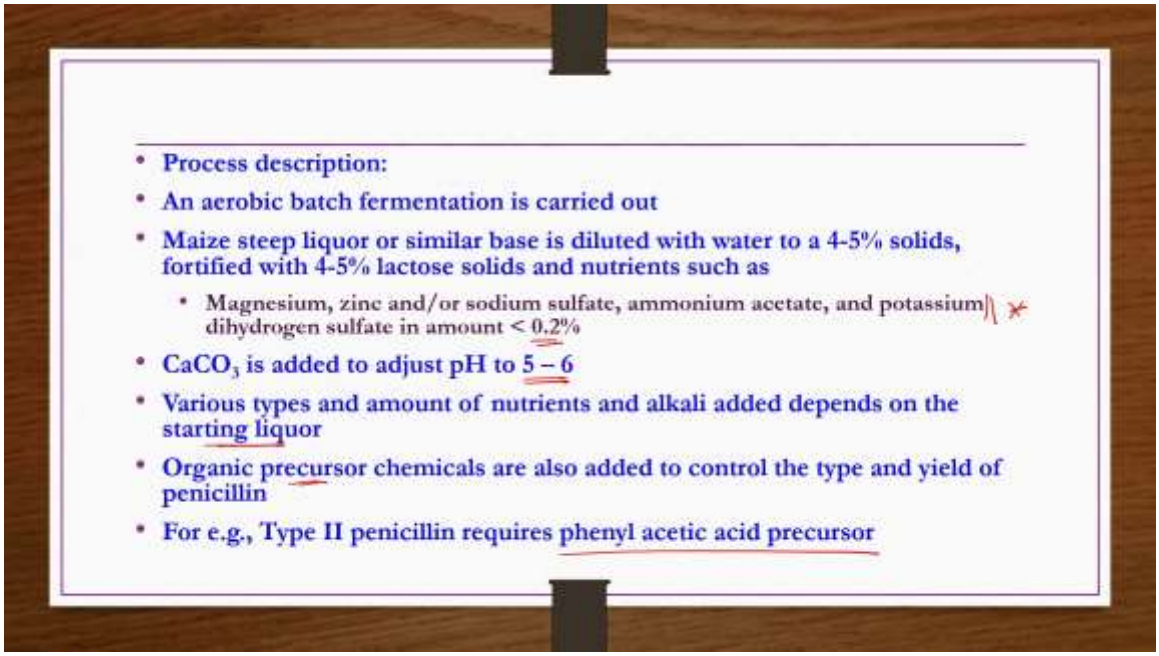

So, whatever details we discussed here in this flow chart those details are also presented here in the text form from the learner's point of view. Anaerobic batch fermentation is carried out maize, steep liquor or similar base is diluted with water to a 4 to 5 percent solids fortified with 4 to 5 percent lactose solids and nutrients such as magnesium, zinc and or sodium sulphate, ammonium acetate and potassium dihydrogen sulphate in less than 0.2 percent amount this is as per the requirement of the type of a derivative that you are producing out of 5 types.

Then calcium carbonate is added to maintain the pH between 5 to 6 various types and amount of nutrients and an alkali added depends on the starting liquor what kind of liquor you have taken. Organic precursor chemicals are also added to control the type and yield of penicillin which type of penicillin you are producing accordingly organic precursor chemical should also be added. Let us say for type 2 that we are discussing you need to add phenyl acetic acid precursor to the whatever the base that you have taken maize, steep liquor or similar base whatever you have taken. So all these steps you do before feeding the base material to the fermenter. After this what you do actually you do the sterilization and then cooling and then once the temperature of the media is 20 degrees centigrade after sterilization and cooling process that you have to feed to the fermenter.

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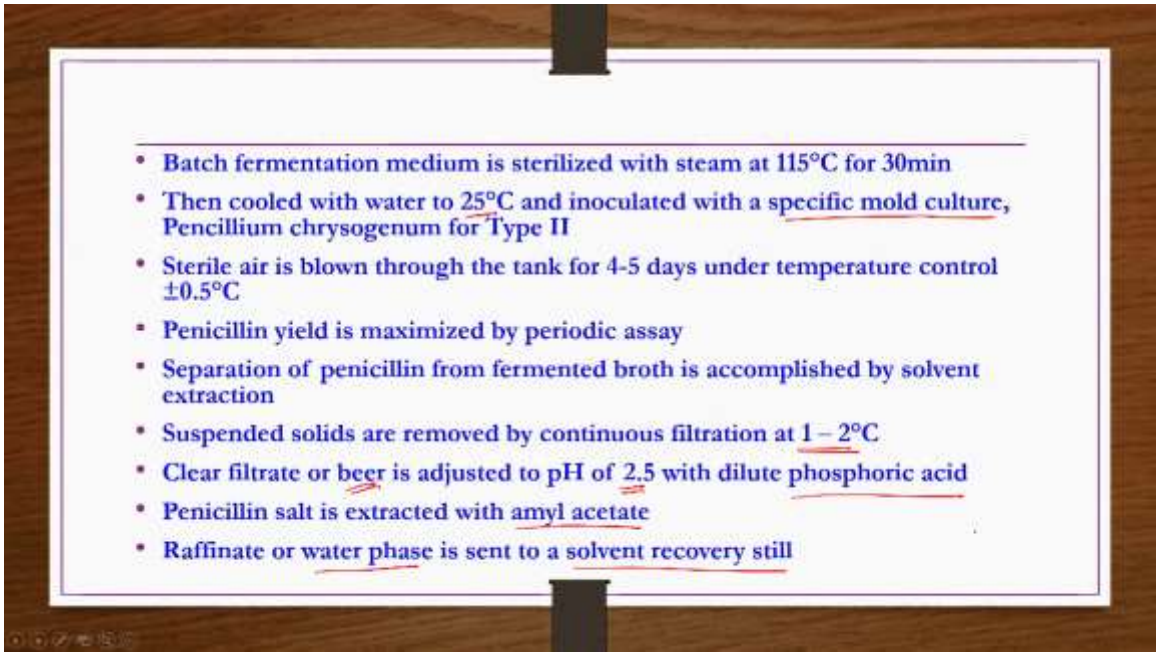

Batch fermentation medium is sterilized with steam at 115 degrees centigrade for 30 minutes then cooled with water to 25 degrees centigrade and inoculated with specific mold culture like you know for type 2 you need to have penicillium, chrysogenum as a you know mold. So that you can make sure that the required fermentation reaction is taking place and and required product is coming out. As we have been discussing last 2 classes also in fermentation the culture has to be very specific, substrate should also be very specific as per the product. Sterile air is blown through the tank for 4 to 5 days under temperature control of plus or minus 0.5 degrees centigrades. Penicillin yield is maximized by periodic assay. Separation of penicillin from fermented broth is accomplished by solvent extraction. Suspended solids are removed by continuous filtration at 1 to 2 degrees centigrade before the solvent extraction. Clear filtrate after continuous filtration is there whatever is there that we call beer is adjusted to pH of 2.5 with dilute phosphoric acid 10 percent phosphoric acid.

Penicillin salt is extracted with amyl acetate in the extractor. Raffinate or water phase whatever is there that is sent to solvent recovery still. Once the recovery is done that is fed back to the extractor.

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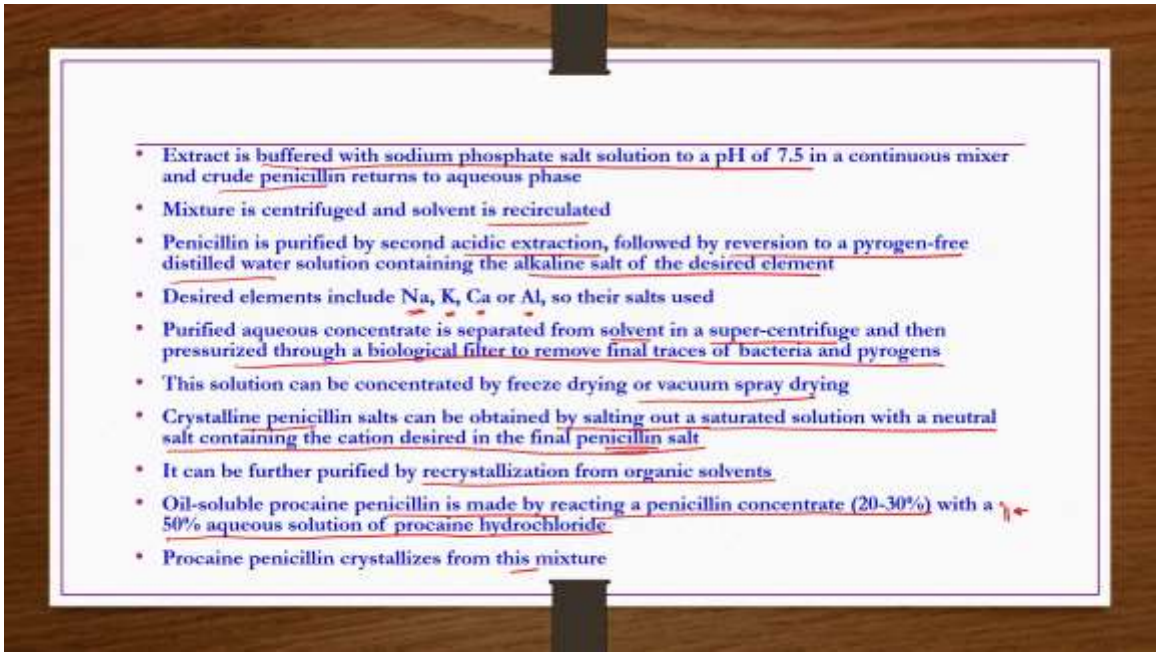

Whereas the extract is buffered with sodium phosphate salt solution to a pH of 7.5 in a continuous mixer and crude penciling returns to aqueous phase.

Mixture is centrifuged and solvent is recirculated to the extractor as shown in the flow chart. Penicillin is purified by second acidic extraction followed by the reversion to a pyrogen free distilled water solution containing the alkaline salts of the desired element. Desired elements include sodium, potassium, calcium, alumina. So, their salts are in general used. Purified aqueous concentrate is separated from solvent in a super centrifuge and then pressurized through a biological filter to remove final traces of bacteria and pyrogens.

This solution can be concentrated by freeze drying or vacuum spray drying as shown in the flow chart. Crystalline penicillin salts can be obtained by salting out saturated solution with a neutral salt containing the cation desired in the final penicillin salt. It can be further purified by recrystallization from organic solvents. Oil based procaine penicillin is made by reacting a penicillin concentrate having 20 to 30 percent with a 50 percent aqueous solution of procaine hydrochloride if you wanted to make oil based. Oil based is required for the injection but slow release of the medicine. Procaine penicillin crystallizes from this mixture.

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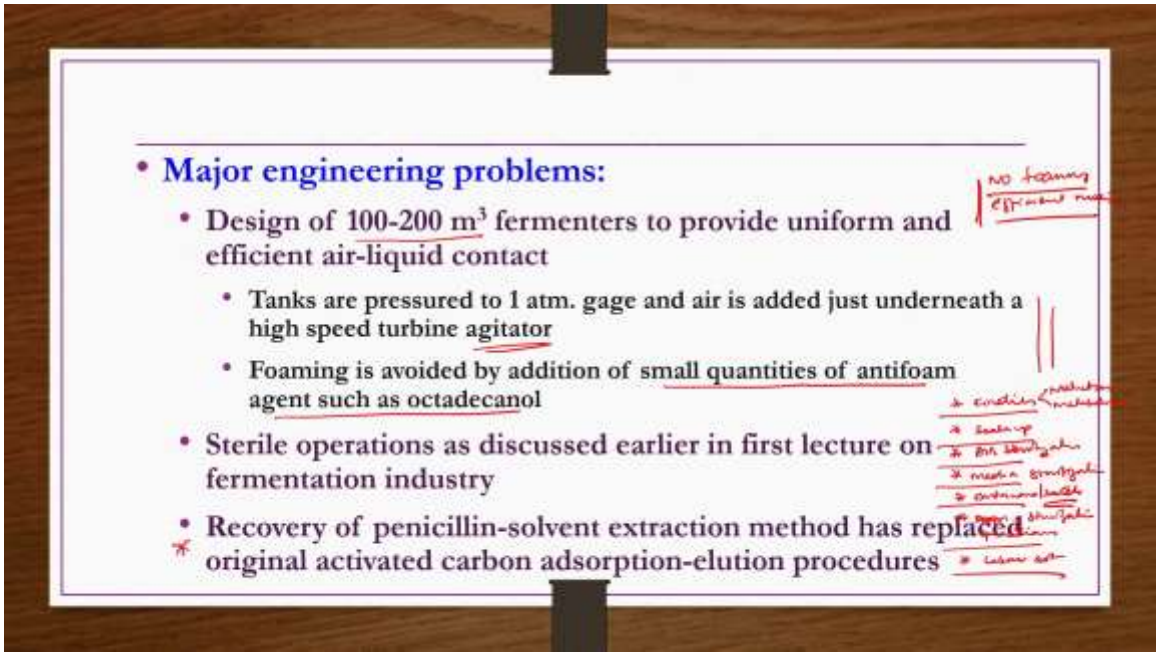

Now coming to the major engineering problems most of the major engineering problems associated with the penicillin production by fermentation process are similar or in fact they are as it is like so called our major engineering problems that we have discussed in the first class of the fermentation industries. Design of 100 to 200 cubic meters fermenters to provide uniform and efficient air liquid contact is really challenging because it is not just designed what it should have there should not be any foaming no foaming and then efficient mixing is also required. So, the scaling parameters you know when we discuss so these things are very essential. So, maintaining broth and then designing such big tanks is really a challenging.

Tanks are pressurized to one atmosphere gauze and air is added just underneath a high-speed turbine agitator. If this air release speed and then turbine speed are not matching or some kind of negative pressure gradients are forming then what happens then these agitators may even break down during the process. So that is the reason it is very essential crucial part of engineering design of the fermenter for penicillin production. Foaming is avoided by addition of small quantities of anti-foam agents such as octa decanol if required. Sterile operations as discussed earlier in first lecture on fermentation industry are same here also let us say kinetics you have to make sure where you know production rate as well as the metabolism rates should be considered then scaling up issue should also be considered some of them are discussed here anyway. And then air sterilization is very much important because drugs you are manufacturing and then media sterilization is also very important and then also continuous versus batch process.

So here most of the things are you know occurring in the batch mode only especially fermentation is occurring in the batch mode though some of the other purification step may be considered continuous as per the economics of the plant. Then design of sterilization operations should also be considered because the entire plant has to be sterilized otherwise impurities may be there and then medicine may not be of sufficient use. Then labor cost, etc. should also be considered while you are doing the continuous versus batch selection. Recovery of penicillin solvent extraction method has replaced original activated carbon adsorption elution procedure anyway.

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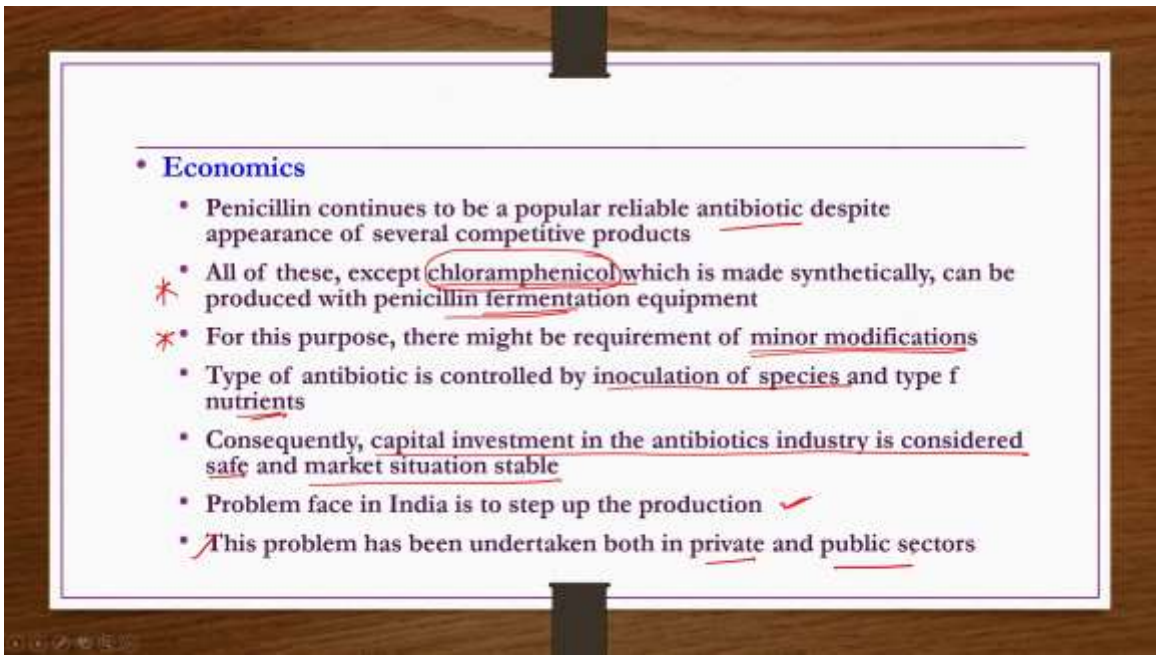

We conclude the lecture with economics. Penicillin continues to be a popular reliable antibiotic despite appearance of several competitive products in the market. All of these except chloramphenicol which is made synthetically can be produced with a penicillin fermentation equipment whatever the setup that you develop for the penicillin fermentation that can be used for all other types of antibiotics as well except this one. So that way at least your capital cost is safe if you are moving from one drug to the other drug because of the market demands. For this purpose, there might be requirement of only minor modifications not major minor required some cases some drugs production there might not be any modification requirements there for this fermentation equipment to produce other antibiotics other than the penicillin.

It depends on the type of antibiotic that you are producing other than the penicillin. Type of antibiotic is controlled by inoculation of species and type of nutrients. Consequently, capital investment in the antibiotics industry is considered safe and market situation stable because of this reason. Problem face in India is to step up the production rate increasing

the production rate but however both of them have been taken care by private and public sectors anyway. So that is all about production of penicillin with this we complete our lectures on fermentation industries.

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References for fermentation industry lectures are provided here. Outlines of Chemical Technology by Dryden edited and revised by Gopal Rao and Marshall third edition. Chemical Process Industries by Austin and Shreve fifth edition. Encyclopedia of Chemical Technology Kirk and Othmer fourth edition. Unit Processes in Organic Synthesis by Groggins fifth edition. However, these 2 books are sufficient enough or the entire lecture notes of fermentation industry are prepared from these 2 reference books. Thank you.