Mass Balance, Heat Balance, Flow Sheet
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Lecture - 2
Mass Balance, Heat Balance, Flow Sheet

Previous class, I introduced the concept of mass balance in a minute operation. Mass balance represents the quantity of liquids that are flowing in with respect to the quantity of liquids, that are flowing out. So, if there is no reaction or if there is no evaporation or loss of liquid whatever comes in should be equal to what ever goes out.

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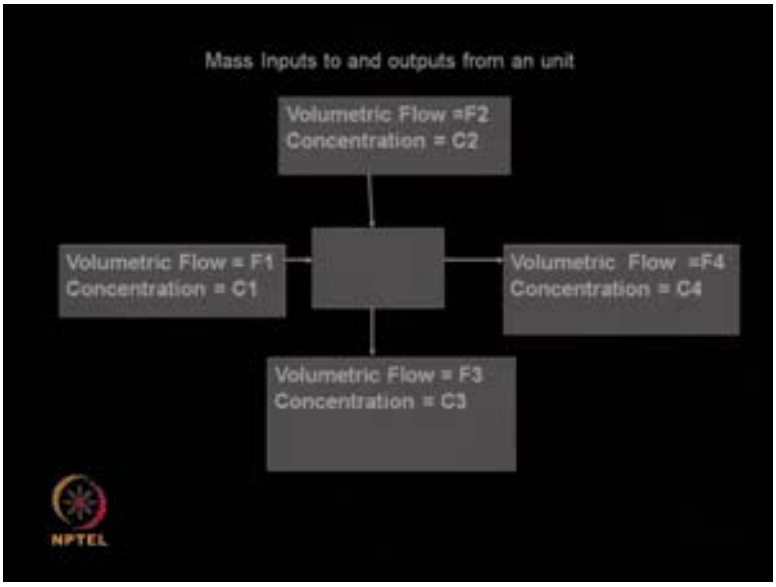

That means; number of streams of liquid that are coming in should match with the number of streams of quantity of the number of streams of liquid that are going out. So, it is very useful to see or to calculate what the Volumetric Flow of Liquid that is; flowing out. So, for example this could be distillation columns you may be having 2 feeds coming in at certain flow rate. And then you can calculate what will be the flow rates of the liquids that are going out, that is 1.

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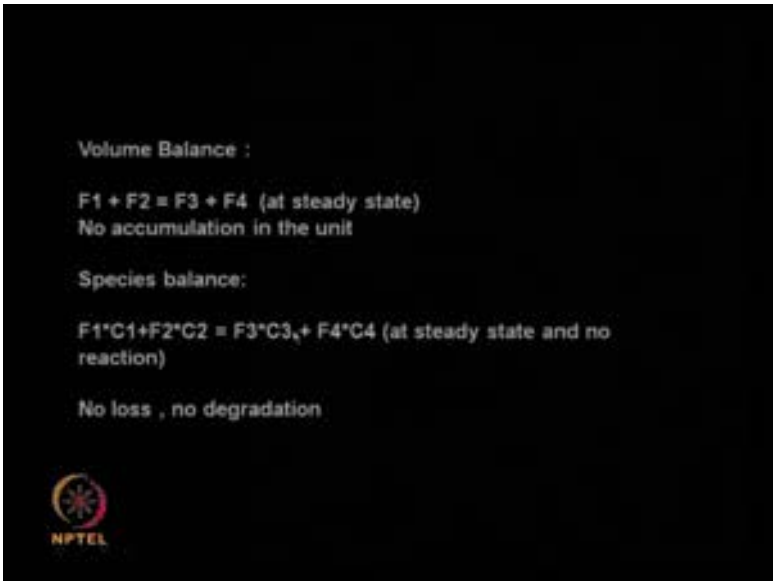

The second point is you can also do a mass balance for a particular species. Imagine there is acetic acid in that stream you can do a mass balance for acetic acid, that is entering the stream is equal to the acetic acid that is leaving the stream, the entire minute assuming that acetic acid is not getting converted to any product. So, the amount of acetic acid entering should match exactly with the amount of acetic acid that is going out. It can be in 2 streams and it can be going out in 2 streams.

But still the total amount should match, assuming that there is no loss or no degradation. And this is done at a steady state, because if you are starting your unit operation when it is in unsteady state condition there will be accumulation inside the unit. So, this numbers will not exactly match, but once it has reached its steady state these should match exactly just like, mass balance.

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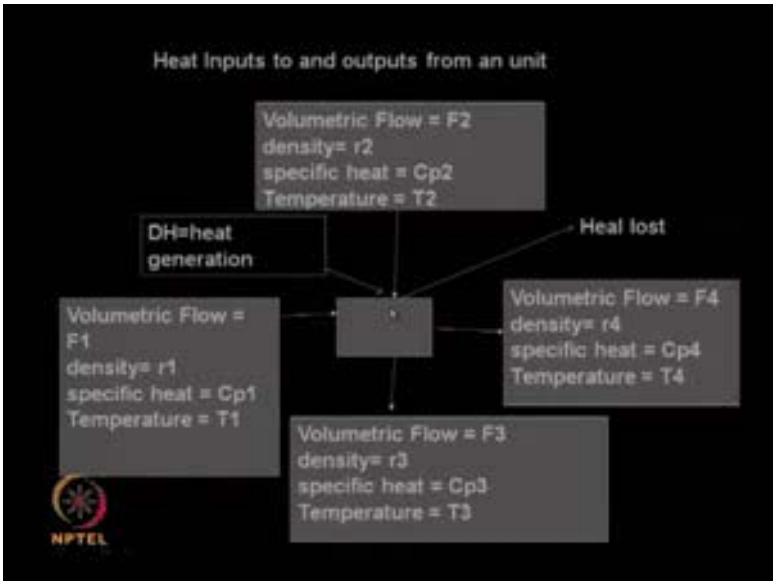

You also have something called Heat Balance, that means; the amount of heat you input, the amount of heat that is going out and the amount of heat generated inside the unit and the amount of heat that is lost to the surroundings they all should be related to each other. For example, if you look at this particular flow sheet you are having 2 fluids coming in. And each fluid has certain amount of heat and there are 2 fluids going out and each fluid has certain amount of heat in them, you are also generating heat.

This heat could be generated because of a reaction or it could be discuss forces. And you are also loosing heat, the heat lost could be because you are cooling the contents or it is lost to the surroundings the unit may be losing heat to the surroundings. So, all these each should balance with each other that means; total amount of input heat should balance exactly with the total amount of output heat. Now, let us look at what is the heat content of a fluid that is coming in? This will be equal to the mass of the fluid that is coming in the specific heat of the fluid multiplied by the temperature corrected at certain atom condition.

So, mcp delta T is equal to the total heat this we have read long time back. Similarly, for this particular fluid it will be the mass that means; flow rate into density, into specific heat, into delta T, that is; the temperature at which it is corrected to at certain dealt value. Similarly, the heat lost will also be the mass that is; flow rate into density, into specific heat, into delta T.

And, similarly, for the 4th stream the total mass is flow rate into density, specific heat into some temperature. Heat generated could be because of exothermic reaction taking place inside. So, you will be knowing what is heat generated inside the reactor? And similarly, heat lost may be because you are cooling the contents or there is a loss to the surrounding. So, once you know that, you can balance all of them together.

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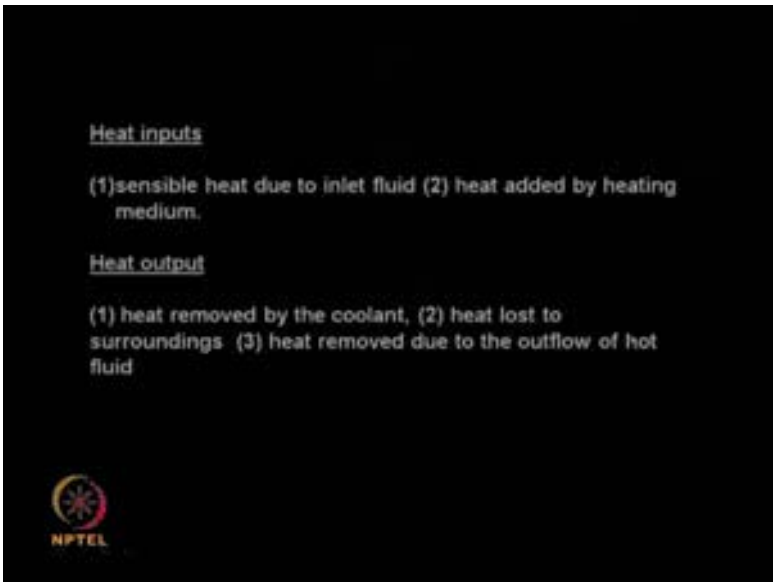

So, the heat inputs as I said is sensible heat due to inlet fluid, heat added by heating medium or exothermic heat. And then heat removed by the coolant, heat lost the surroundings or heat removed due to the out flow of the fluid. The outflow of the fluid is like this these 2.

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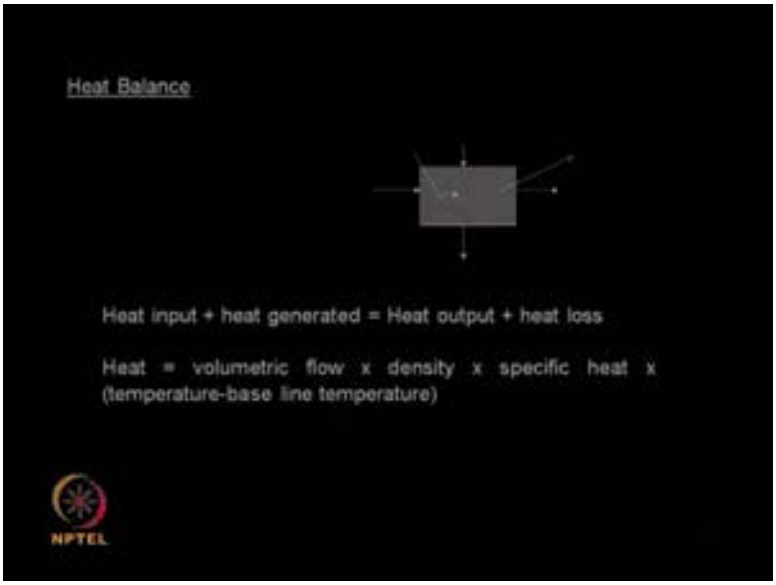

So, when you do a heat balance what you do? You say all the input heats that is; on the left hand side of this equation should match with all the output heats that is; on the right hand side. That is; the heat input, heat generated which is equal to heat output, heat lost. So, how do you calculate the heat? Volumetric Flow rate into density give you the mass flow rate, into specific heat, into delta T. How do you calculate delta T? That is a temperature or the fluid minus certain base line temperature that is the delta T. So, that is how you do a mass balance? I am sorry, how you do the Heat Balance? Heat Balance is very, very important to find out how much heat I need to put in to heat contents.

For example, if I am trying to increase the efficiency I would like to heat, if it is an exothermic reaction you would like to cool. So, I would like to know how much coolant I need to pass. So, for all these calculations I need to do something called the Heat Balance .So, in each of the units we design in a downstream. We need to carry out both mass balance and heat balance to see am I having any losses in the mass or am I having any losses in the heat or the amount of heat required to raise a temperature of the contents of the fluid.

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Now, let us look at few flow sheets relate to downstream. And I will try to focus on the important salient points what types of non-stream units are used? And what are the costs involved in each one these downstream? So, lets us take a few examples; it just gives you an overview of how we have typical flow sheet we look at?

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I just introduced about a fermentation of sugar for production of alcohol. Let us look at the same thing again generally; yeast is used in the industrial scale for producing Ethanol. So, you have sugars if you take countries like, Brazil or India they use cane

sugar for production of alcohol. If you take U S A normally; corn is used for production of alcohol. There are different types of these species which are used for the fermentation cerevisiae this is; generally, used in industrial scale. The raw material cost takes care of almost 70 percent of the cost of ethanol production. So, if you can reduce the raw material cost you will be able to reduce the production of ethanol.

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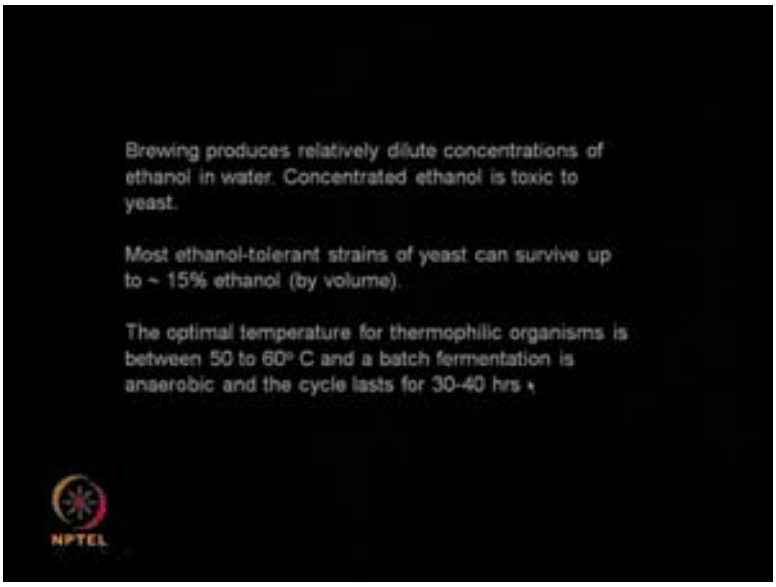

The main problem with these organisms are they are not very tolerant to ethanol, that means; they are tolerant to only maximum there are certain genetically, modified yeast species which can take up to 15 percent ethanol. But generally, 8,9,10 percent of ethanol the fermentation is stopped because the organisms are not tolerant. So, you see that they broth after fermentation is extremely dilute. If it is 10 percent, 90 percent contains water and other bio mass, dead cell, debris and salts, so in each to recover the ethanol of only 10 percent from this large amount. So, that is the main reason it produces very dilute concentration of ethanol.

The optimal temperature for thermophilic organism is about 50 to 60 degree centigrade. So, because these organism have genetically, modified they are able to be more tolerant towards ethanol number 1 number 2. They also can be operated at slightly higher temperature as you can see almost 60 degrees see we can operate. And the fermentations is generally, done in batch mode and it takes about 40 hours for the entire fermentation process.

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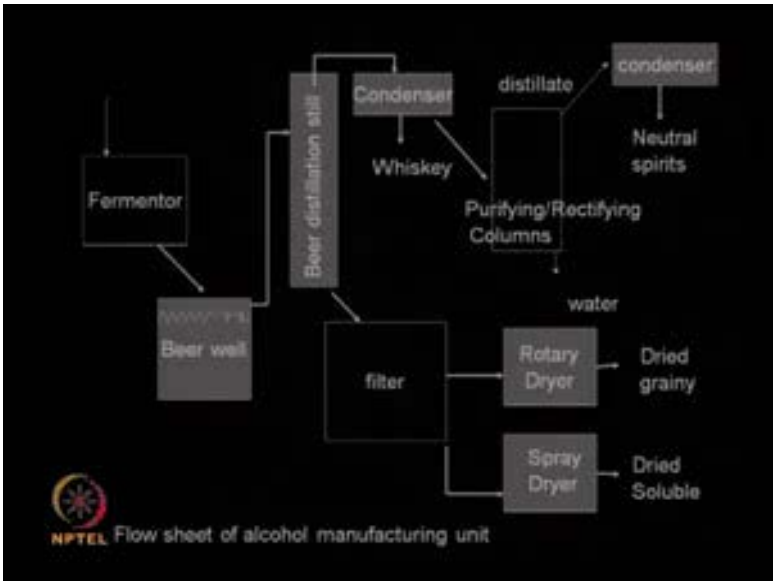

Once, you have done the fermentation and you are talking about 10 percent ethanol you need to purify to almost 95 percent. So, obviously; you need to do lot of distillation that is where distillation is there. And initially you have very large distillation column which will increase the ethanol amount from 10 percent to almost 30, 40 percent. And then finally, to a large number of distillation column which raises up to 90 percent.

One important point if you will remember ethanol water forms an isotropic mixture, that is; you cannot purify the ethanol beyond 89 percent unless you have certain isotropic separation techniques. If you want to reach up to 99 or almost 100 percent ethanol So, isotropic distillations involve adding a 3rd component, adding an entrainer, so that the isotope is broken or sometimes you add an absorbent so that the isotope is broken.

So, if you want 100 percent ethanol you need to resort breaking of this isotope that is; on the right hand side here actually. Now, if you look on this side you have the biomass so you need to filter the biomass and then you can dry the biomass. And it still contains certain proteins which can be used as animal feed here. So, once you dry the biomass it can be sent for animal feed here.

So, this is a typical downstream for the manufacture of ethanol from sugars. So, most of the downstream unit as you can see distillation columns and filtration units and because ethanol is tolerant to high temperature they can resort to distillation column. But if you are going for protein or if you are looking at bio molecules they may not be tolerant for

such high temperature. So, some of the companies involved in the manufacture of ethanol this is; 2007 data. So, you may have more companies coming into it.

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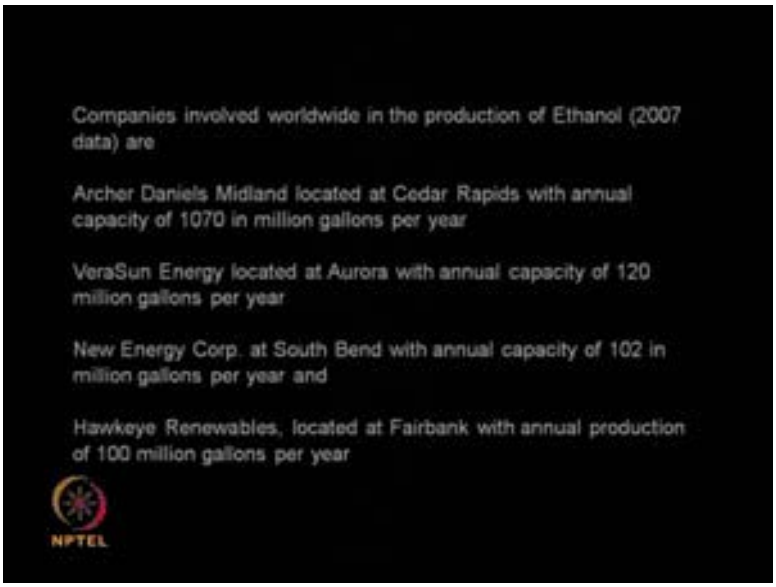

It just gives you an idea of what types of companies are involved? As you can see Archer Daniels they produce 1070 million gallon per year of ethanol. There are Sun Energy systems that are producing about 120 million gallons. New Energy co-operation about 102 gallons another Hawkeye Renewables are producing about 100million gallons per year. So, you will talk about in terms of 100 of millions of gallons of ethanol per year. Ethanol is not only used into for making alcohol industrial applications as well as for consumption purposes actually.

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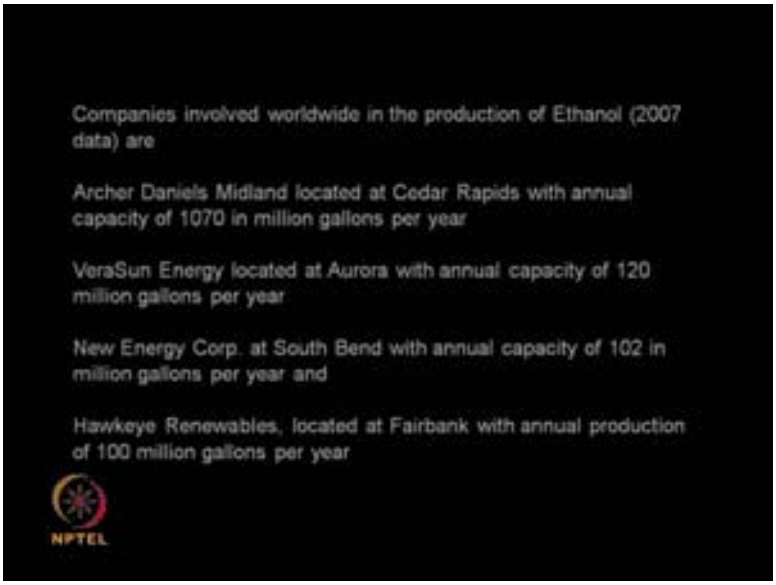

Let us, look at next product it is; the Amino Acids. Amino Acid is a huge business multi-billion dollar business. Because they are used in animal feed, in flavor enhancing, in medical product, as a food additive and for even improving the energy processes. So, if you look at lysine, methionine, and threonine they are used in animal feed. Flavor Enhancers monosodium glutamic system acids. I think we are all used to this monosodium glutamic used in Chinese food and flavoring serine, aspartic acid they are all flavor enhancer. Look at glutamic acid, lysine, methionine we are talking in terms of about 0.5 to 1 million tons per annum production is a 200 data.

So, you may be adding about 10, 15 percent much more than that actually. So, the first 2 are made from fermentation and this is made by chemical synthesis actually. And L-aspartic acid and L- alanine are made through enzymatic synthesis. So, when you say enzymatic you are talking about a bio transformation. So, these 2 are made by fermentation. And this is made through chemical route and these are made through bio transformation.

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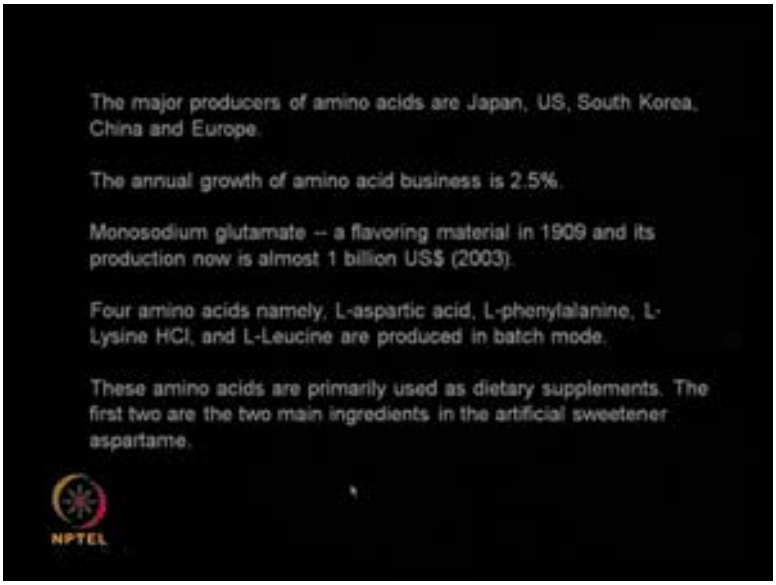

So, the major producers of amino acids Japan, US, South Korea, China and Europe; so they are talking about an increase of about 2.5 percent annual growth. Monosodium glutamate we are all use to it is a flavoring material. And it was introduced in the year 1909 and the business is about 1 billion USS dollar that is a big business which is used in flavoring Chinese food L- aspartic acid, L- phenylalanine L- lysine hydro chloride, L-Leucine they are all produced in batch products actually.

All these also are used in dietary supplements they are used as artificial sweetener. So, the first 2 are used as artificial sweetener aspartame again that is a very big business with more people having diabetic and glucose intolerance. These artificial sweetener businesses are also becoming big.

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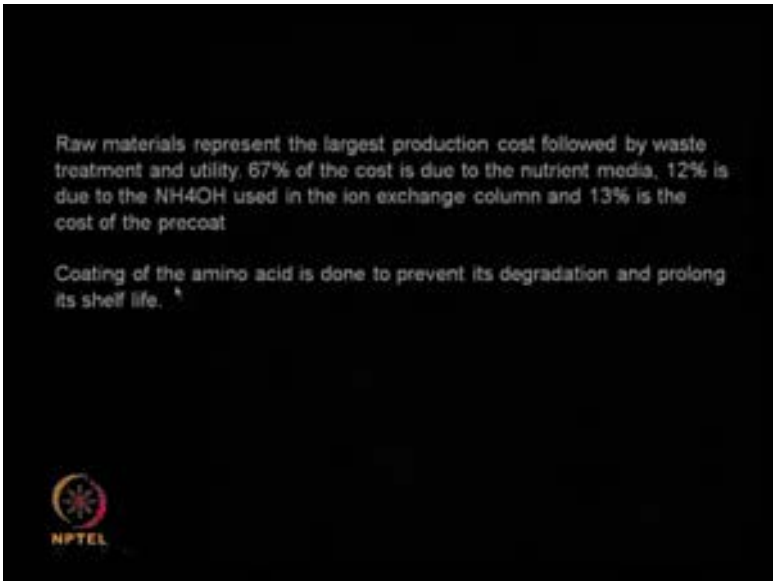

Raw materials contribute the largest in the production cost and remaining is waste treatment and utilities. So, 67 per of the cost is due through the nutrient media, 12 percent is because of ammonium hydroxide used in ion exchange column, 13percent is the cost of pre coating. Why do you pre coat? You want pre coat all these amino acids because you want to prolong its life, you want to prolong its shelf life, and you want to prevent degradation. That is why you need to pre coat these amino acids and about 13 percent of the cost is because of the coating of these amino acid products.

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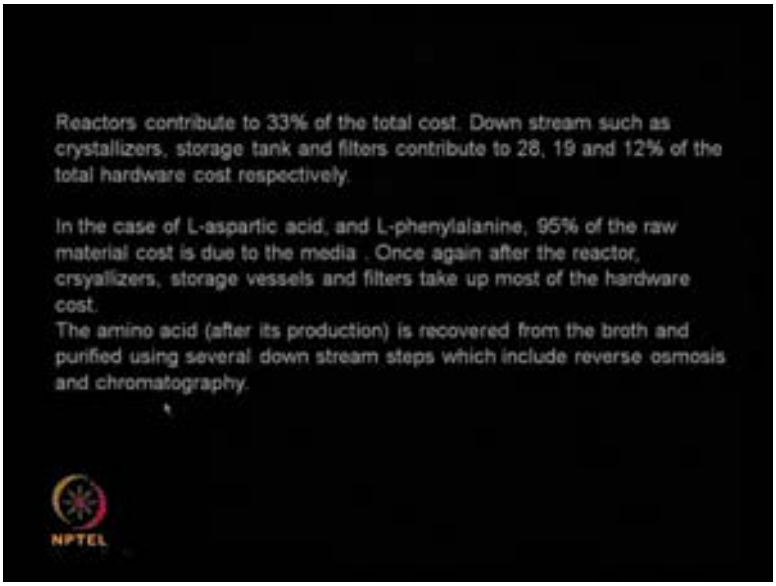

If we look at the hard ware side we looked at the chemical cost; now, let us look at the hardware side reactors contribute 33 percent of the total cost. And then if you look at the downstream side remaining 67 percent crystallizers, 28 percent of the hardware cost is because of crystallizers, 19 percent is because of storage tank and12 percent because of the filters.

So, you see once you know the contribution of cost by each of the hardware unit in the downstream if you can reduce the size of the unit; obviously, you are able to reduce the cost of the entire plant. In L- aspartic acid as well as L- phenylalanine production 95 percent of the raw material cost is due to the media. So, if you can optimize the media efficiently you can reduce the operating cost in the manufacture of L- aspartic acid and L-phenylalanine.

Once again reactors crystallize storage tanks and filters contribute the maximum and the overall hardware cost. Let us look at the downstream the amino acid after its production is recovered from the broth using several downs streams steps. You also use something called reverse osmosis and you also use something as chromatography. We are going to talk about each one of this downstream unit in more detail in the forth coming classes actually.

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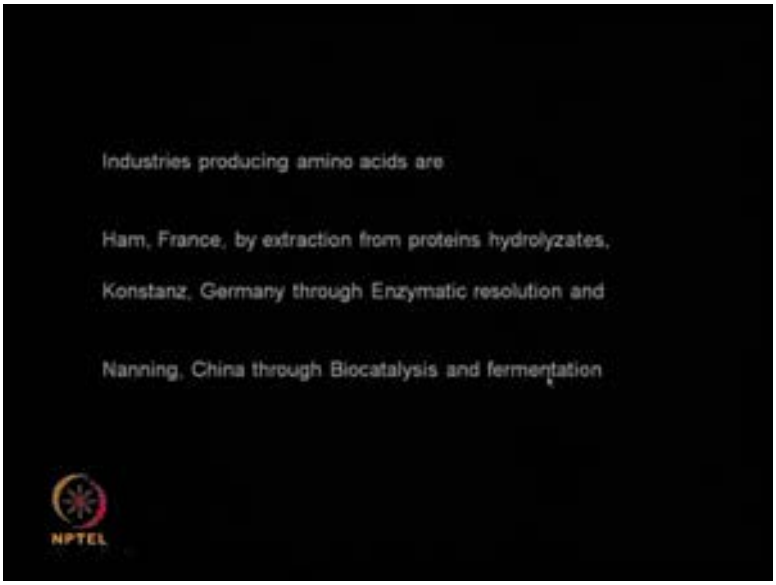

Industries which produce these amino acids companies in France, companies in Germany, companies in China some of them using protein extraction, enzymatic

resolution, Biocatalysis, fermentation product.

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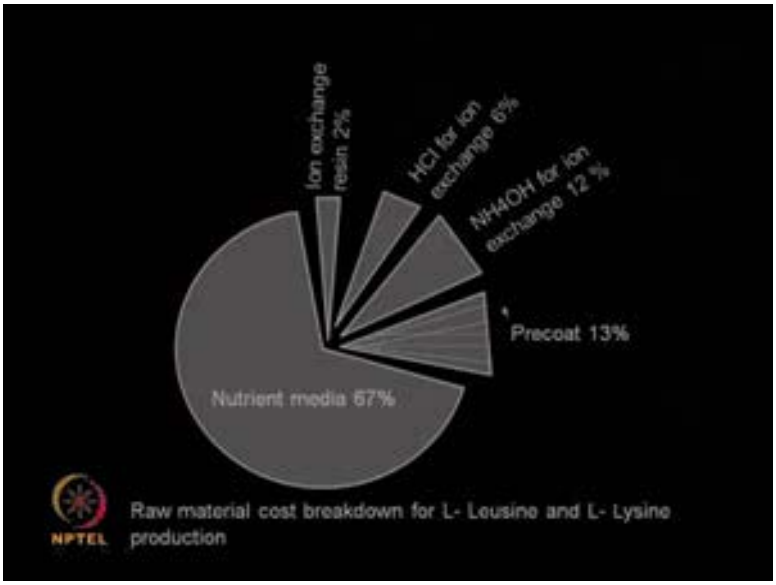

So, this is a picture which tells you the raw material cost break down for the manufacture of L –Leusine and L- lysine. So, 67 percent of the cost is because of the nutrient media; so you can focus on optimizing the nutrient media. A typical nutrient media will contain a carbon source, a nitrogen source, micro nutrients, salts, metals and so on. So, if you can reduce the usage of the obviously you can impact this cost.

Next comes the use of ion exchange ammonium hydroxide; so if you can reduce the usage of ammonium hydroxide obviously you can impact here. Next is again hydrochloric acid which we use in the ion exchange for re generating the ion exchange; so if we can reduce the usage of hydrochloric acid you can impact on this particular cost. Then finally, a large amount of cost is incurred in the pre coat. So, if you can optimize on the pre coating of these amino acid, you can impact on this particular area.

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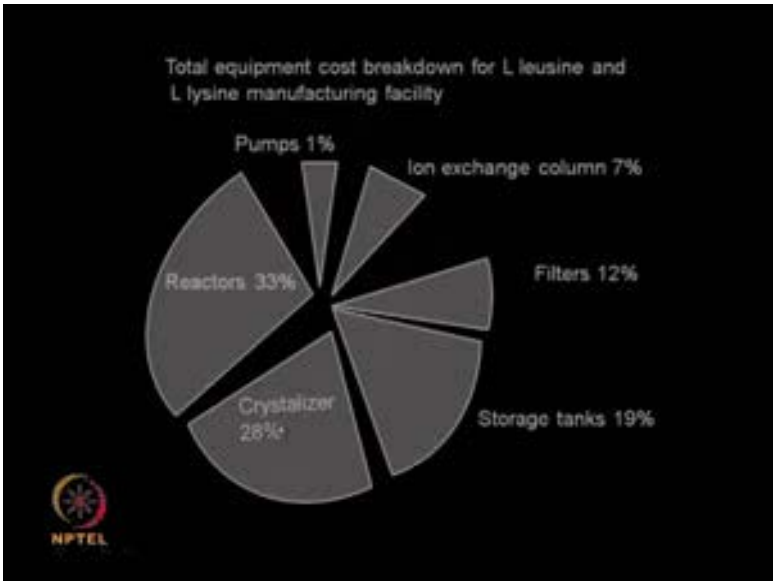

The total equipment cost in the manufacture of L -leusine and l- lysine 33 percent is because of the reactors. So, if you can bring down the reactor side you can reduce the number of reactors obviously, you can impact here, And then again 28 percent is the crystallizers, 19 percent is storage, 12 percent is the filters and 7 percent is the ion exchange. So, one can look at how to reduce the size of the filters? How to reduce the size of the storage tank? And how to reduce the size of the crystallizes?

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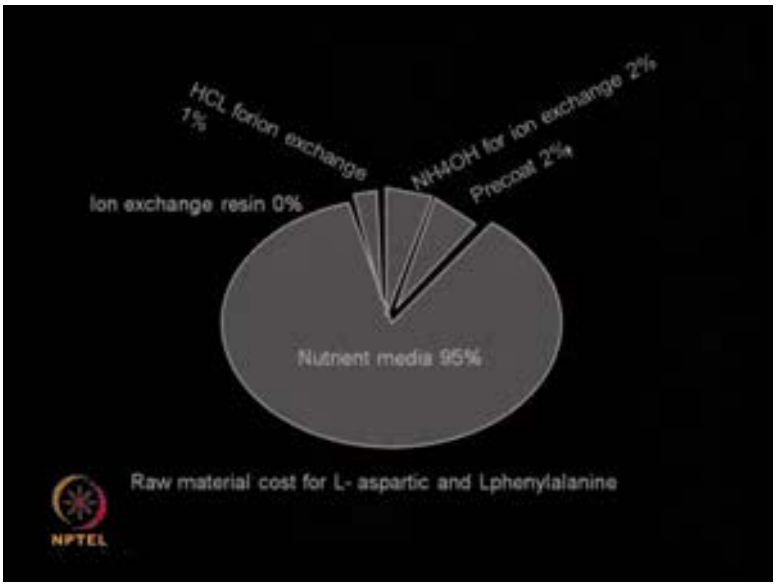

If you look at the aspartic acid and L- phenylalanine large amount of cost is because of

the nutrient media. So, one needs to focus on needs to do more r n d and trying to optimize the nutrient media you can see 95 percent of the cost. So, one there is lot of scope to reduce the cost of the raw materials in the manufacture of L -aspartic acid and L phenylalanine. If you can try to focus on the various components the nutrient and try to reduce the amount of nutrients used here. And 2 percent of the cost is because of the ammonium hydroxide usage in the ion exchange ((Refer Time: 18:53)).

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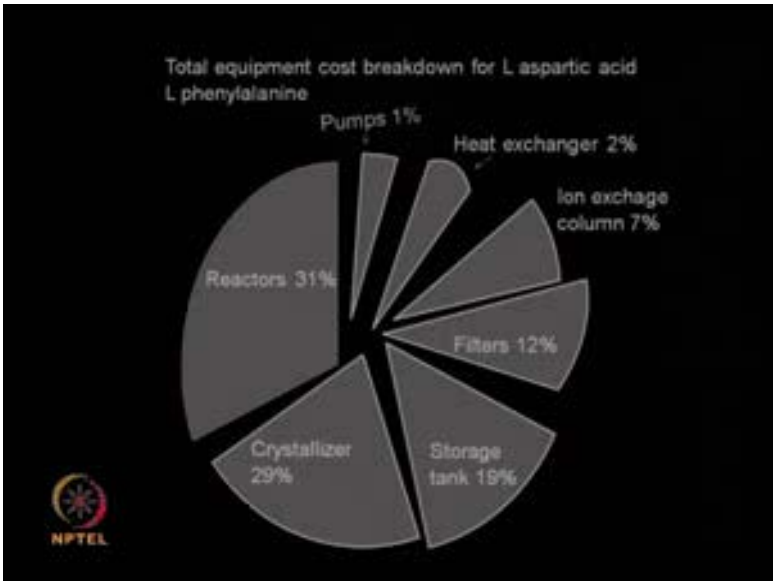

Again, if you look at L-aspartic acid, L- phenylalanine reactors take up 31 percent of the cost, crystallizers take up 21 percent of the cost, storage tank takes up 19 percent of the cost and filter takes up 12 percent of the cost. So, reducing the size of the filter, storage tank and crystallizer one can really bring down the total equipment cost in the manufacture of aspartic acid and phenylalanine.

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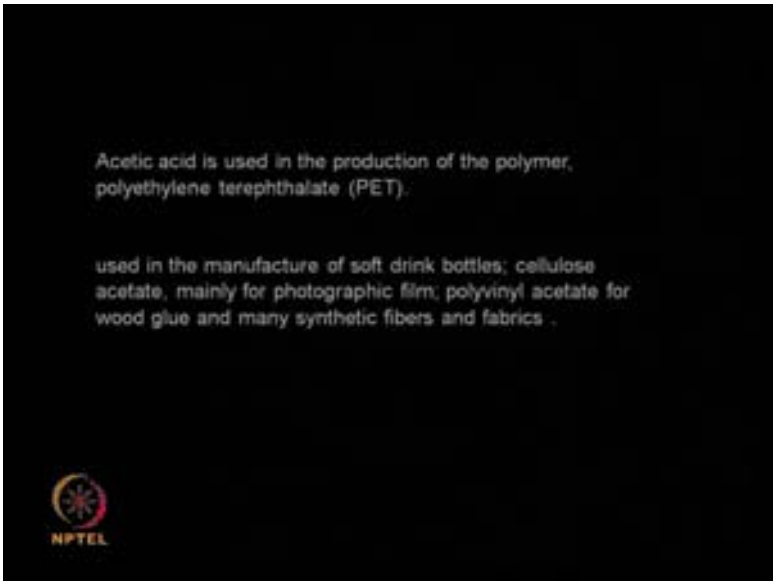

Now, let us look at the next product acetic acid is used in the production of polyethylene terephthalate PET. That is a polymer used widely almost all the soft drink bottles or manufacture using this polymer cellulose acetate which is used in the photographic film. Polyvinyl acetate which is used in the glue and many synthetic fibers they all have this particular polymer percent in it. And this polymer is made from acetic acid. So, acetic acid has very large usage in large number of polymeric products.

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So, acetic acid can be manufactured both by synthetic root as well as the bacterial

fermentation. For example, vinegar that is another name which we use quite frequently and quite a lot in food is also made, but it is made using a biological route. Vinegar about 10 percent of the world production is vinegar and that is made using biological route actually. So, most of the world food purity laws requires that vinegar should that is; made in for food application must be based on the biological route.

So, that means if you want to make a polyethylene PET. The acetic acid can be made through chemical route, but if you are interested in manufacturing vinegar than the acetic acid has to be manufactured using biological methods. So, most of the acetic acid in the chemical route is based on the methanol carbonylation. So, it is a homogeneous process using methanol as the raw material and it is a carbonylation reaction. So, the carbon monoxide is put inside methanol to make the acetic acid.

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If you look at the bio chemical route for manufacture of vinegar we use bacteria genus called Acetobacter. Even certain genus of clostridium can also be used for making acetic acid the advantage here is, it does not have to through ethanol because once you want to go through ethanol. Ethanol is very toxic for many organism and many organisms are not able to survive in the ethanol medium. So, clostridium is another organism which can convert the sugars into acetic acid without going through ethanol in between.

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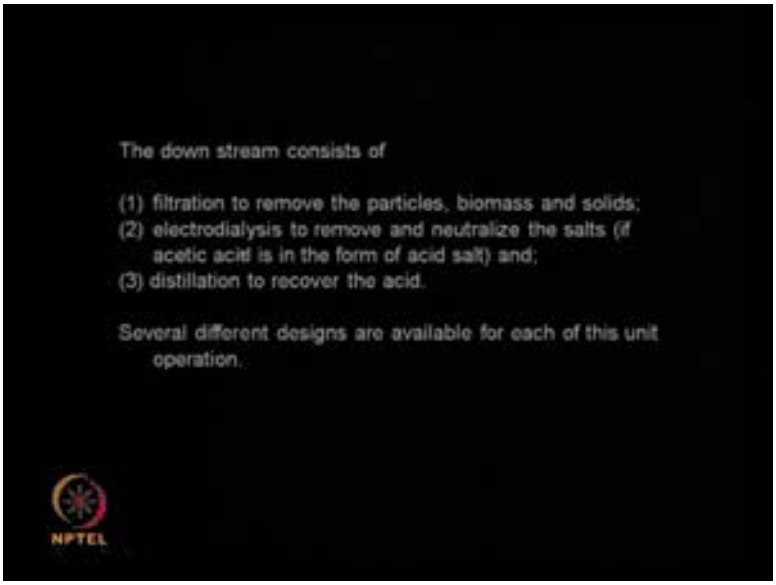

So, what are the down streams? So, once you make the acetic acid you have to filter the media. So, for removing the particles, biomass and solids and then you resort to electro dialysis to remove and neutralize the salts that are present because you are going to have lot of salts present. So, that the acetate becomes acetic acid and then finally, you resort to distillation to recover the acid.

So, these are the 3 downstream steps in acetic acid manufacturing; initially, the filtration to remove all the biomass, cell debris, solids. And then using an electro dialysis you convert the acetate into acetic acid and then finally, you have the distillation. So, many different ways of filtration can be resorted to; so many different ways of electro dialysis can be used resorted to and so many different ways of distillation also can be followed.

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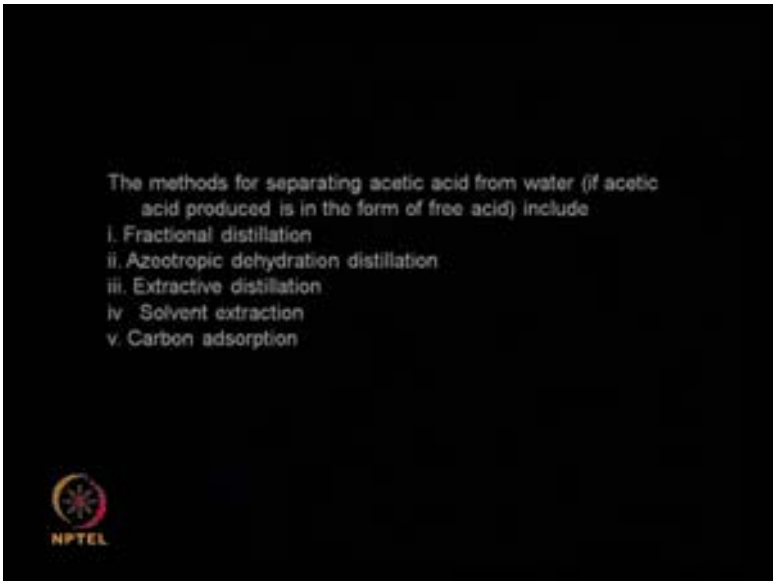

So, if you are to separate the acetic acid from water you can use fractional distillation, isotropic distillation, extractive distillation. So, these are 3 different types of distillation or you can use a solvent; so that the acetic acid alone is extracted from the dilute media you can also resort to absorption. So, carbon can be used for absorbing the acetic acid. So, all these different techniques can be used and each one has got advantages disadvantages, there is also a cost factor involved some of them are expensive at certain scales, some of them are cheaper at certain other scales and so on. So, one needs to decide depending upon the cost factors.

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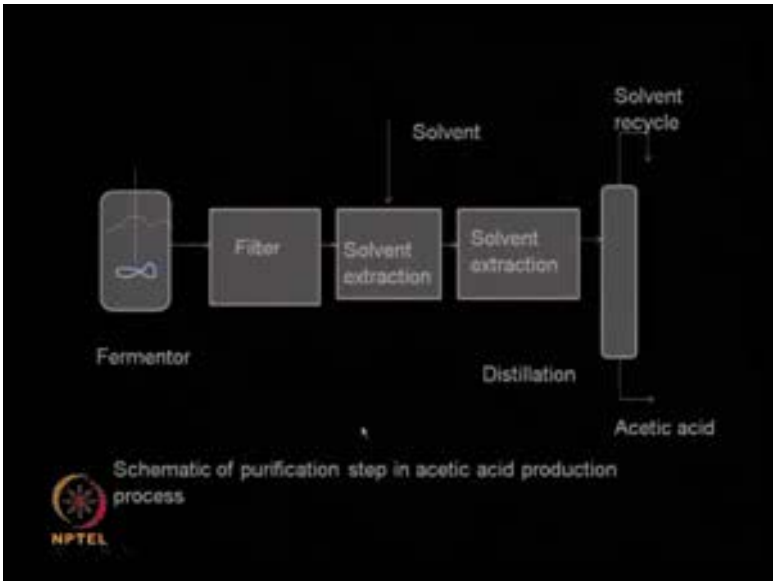

So, this is a typical flow sheet for the manufacture of acetic acid. So, the fermentation takes place here and then you have the down streams. So, you have filter solvent extractions; so many solvent extractions and then the distillations. Then the solvent is coming here, which can be sent back into the solvent extraction. So, the distillation will produce acetic acid. So, this is a typical acetic acid manufacturing plant this is how it looks like actually.

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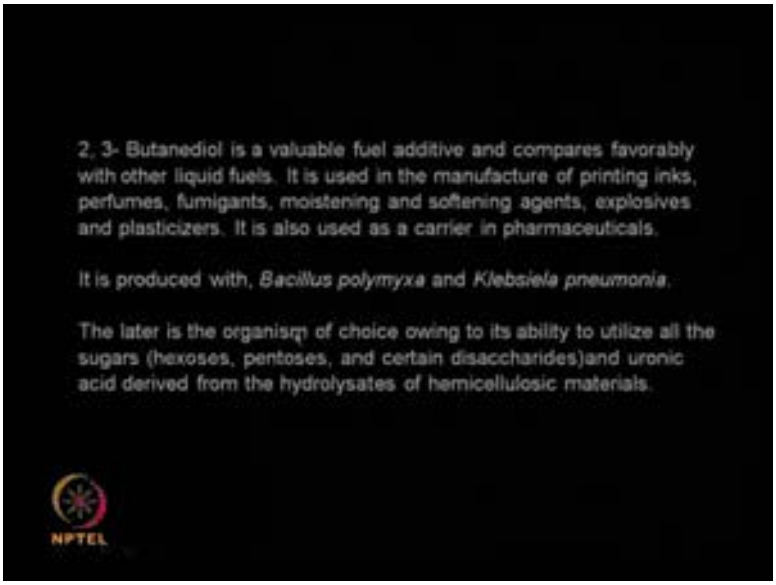

Let us look at the next product it is a 2, 3 Butanediol. 2,3 Butanediol is a valuable fuel additive and it compares very well with other liquid fuels it is also used in printing inks, perfumes, fumigant, moisturizing and softening agents, plasticizer in explosives also. And it has got some applications in pharmaceutical as well as a carrier drug molecule. So, it is widely used in a wide range of products. And it is produced using these 2 microorganisms. Generally, the second organism is preferred because it can utilize a large number of sugars for converting into 2, 3 Butanediols. So, hexoses, pentoses or even disaccharides can be used which can be fermented to 2, 3 Butanediol.

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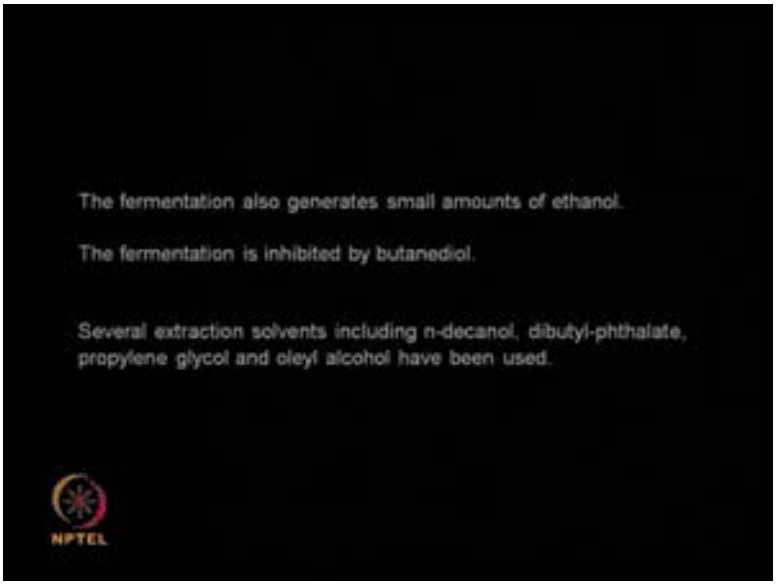

The fermentation also produces little bit of ethanol that is one point. So, ethanol Tolerancy is very very important number1, number 2 Butanediol itself is toxic to these organism. So, what you have to do is as soon as you produce Butanediol you have to remove the Butanediol and then send, and then brought back into the reactor for further fermentation.

So, extraction is part of the fermentation here several solvents are used for extracting Butanediol it includes say n-decanol, dibuty,l-phthalate, propylene, glycol and cleyl alcohol. So, you can use different solvents, each solvent has different affinity to Butanediol or partition coalition to Butanediol and the yield of Butanediol into the solvents depend upon the operating conditions also.

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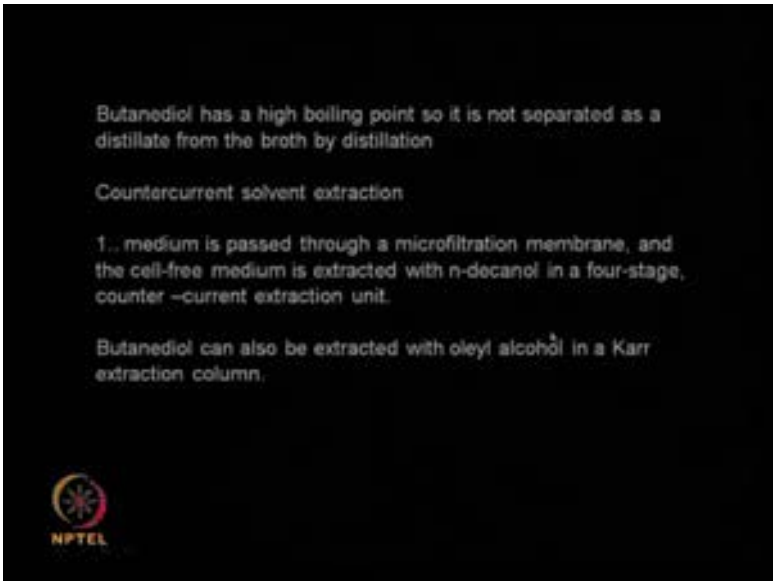

So, Butanediol has a very high boiling point. So, you cannot use a distillation column and hope to get Butanediol at the top of the distillation assembly. So, that is the main problem; so extraction is the only way of extracting Butanediol after the fermentation. So, how do you do it? First the medium is passed through a filtration membrane so once it passes through the cell free medium is obtained.

And then you use a solvent for extraction like for example, n-decanol you can use a 4 stage counter current extraction. So, what does counter current? It means the broth which is free of cells pass in one direction and the extracting solvent like, n- decanol possess through another direction, that is; what is called counter current. So, Butanediol is extracted using a 4 stage counter current you can also use Karr extraction column for extracting Butanediol. Here oleyl alcohol is used as a solvent.

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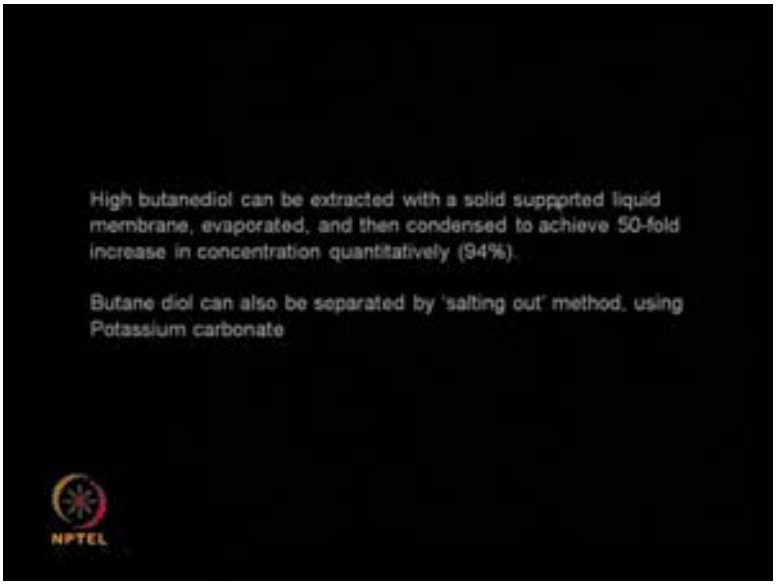

You can also use solid supported liquid membrane for extracting and then later on you can remove the liquid membrane. So, that the Butanediol is extracted. So, this is very good process you can extract almost 95 percent 94 percent of the Butanediol. You can also use a salting out technique by adding potassium carbonate. So, I add potassium carbonate so because of the changes in this solubility Butanediol precipitates out.

So, that is another technique so you see so many different approaches can be used we can use extraction counter, current extraction ,solvent, n-decanol or oleyl alcohol with a Karr extraction column, liquid membrane or salting out technique by using potassium carbonate as the salting out material. So, each one can be used and each one gives you a different amount of extraction efficiencies.

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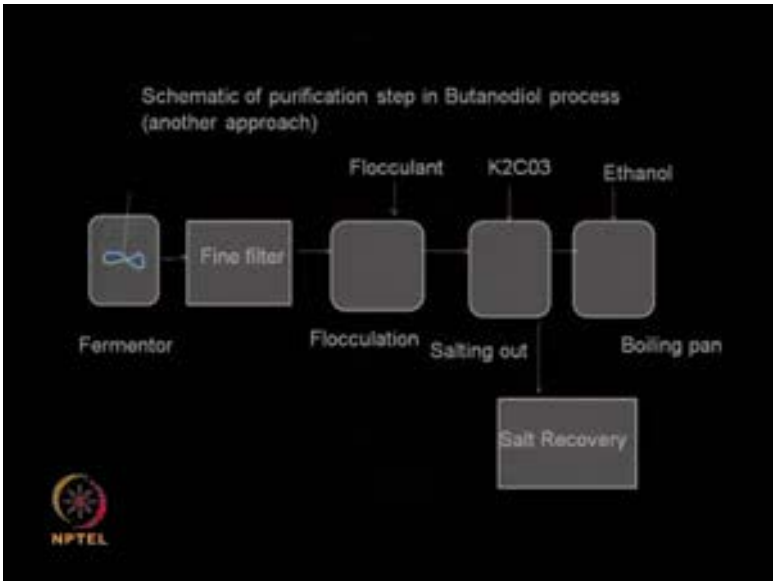

So, a typical schematic for the Butanediol process you have the fermentor on the left hand side. Then immediately, after the fermentation you have a micro filtration to remove the cell and biomass which can be put back into the reactor because they are active biomass. So, it can be put back and then once you get the cell free liquid you can resort to extraction. And the solvent can be removed from Butanediol using a distillation column and the solvent can be put back into the solvent extraction in it.

So, you can have a closed system like that solvent is put inside in a counter current manner and the solvent, Butanediol can be put inside a distillation column. Solvent can be extracted I mean, distilled from the top of the distillation column and again put back into the extraction Butanediol can be collected here. There is going to be waste also whatever solids or unwanted metabolites that is; constitutes the waste. So, this is based on solvent extraction system. But if you are interested to salt out then obviously, what do you do? You need to add potassium carbonates so that Butanediol comes out.

So, here in this approach what do you do? You have filtration so that the solids are removed then you flocculation which will remove floating solids then you add potassium carbonate. Because of the difference in the solubility Butanediol settles out and then we can take out my product here. So, 2 different approaches one is based on extraction, another is based on salting out which I showed you. So, each of these approaches require different types of downstream steps.

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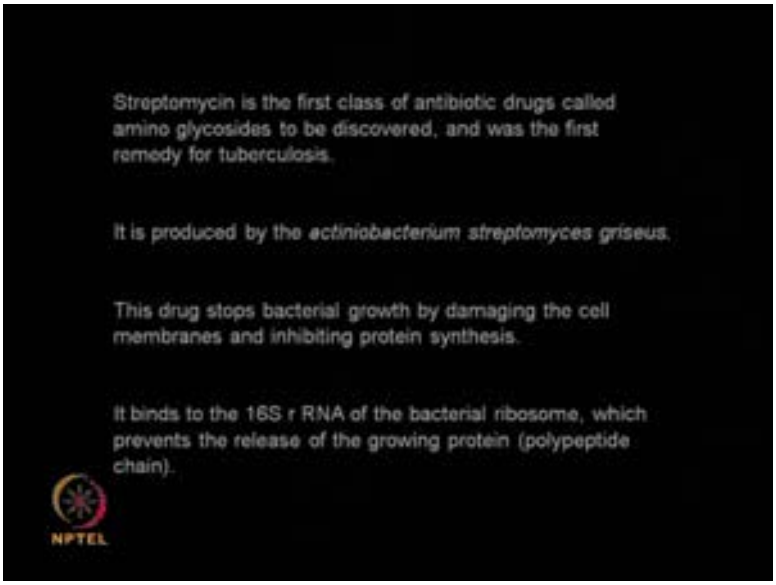

So, companies which manufacture Butanediol you can see some American companies, German companies, Swiss companies even Chinese companies are there. They all are involved in the manufacture of Butanediol lets go to antibiotics streptomycin. This is first class of antibiotics that means; it came almost 20years back these are based on they are called amino glycosides they are the first remedy for tuberculosis also it is produced by an organism streptomyces griseus.

This drug stops bacterial growth by damaging the cell membranes hence, the protein synthesis it binds to this16S r RNA of the bacterial ribosome. And hence; it prevents the growth of the protein, so; this is the more of action of this particular antibiotics streptomyces. And hence; it was used quite a lot in tuberculosis even now; it is being used for treating tuberculosis. So, it is still got quite a lot of application it is an antibiotic.

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Many drug companies manufacture streptomycin, Merck and co. Ranbaxy labs in India Pfizer Glaxo Smithkline, Novartis. So, you can see large numbers of Pharma companies are manufacturing streptomyces. Because it is, still use as anti-bacterial agent, in the treatment of tuberculosis.

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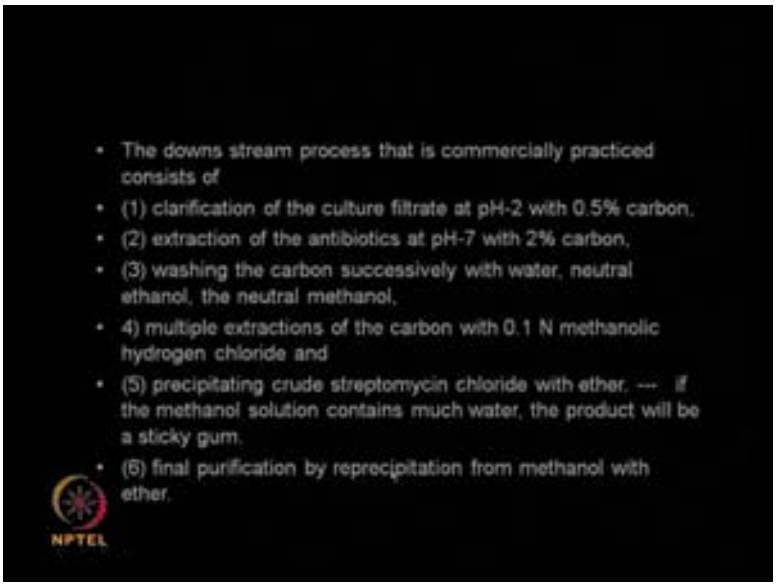

So, what are the downstream steps that are commercially practiced? First is the clarification of the culture filtrate. So, clarification means, you are removing the flocculants so it is based on pH adjustment you and it uses carbon to absorb the

unwanted metabolites and waste. Then you are resorting to extraction at a pH of 7 then you are washing the carbon with water, neutral ethanol, and neutral methanol. So, you keep on washing until whatever streptomycin has been absorbed on the carbon is collected. So, that is why you resort to multiple extractions once you do that, you are precipitating the crude streptomycin chloride with ether.

If it contains water it going to be a sticky gum so you need to remove as much water as possible using ether. And finally, you are purifying this streptomycin with ether so these are the various downstream steps. First you are clarifying means, you are removing the floating solids and then you are extracting and then washing the carbon several times with water, ethanol, and methanol.

And then you are adding hydrogen chloride and then precipitating it out and then finally you are purifying using ether from the methanol. So, this leads to a pure product of streptomycin. Product purity is very, very important in this particular case because you are talking about a drug. And anything that needs to be consumed by human it has to pass through the F D A, that is; the food and drug authority USA which stipulates certain conditions for purity.

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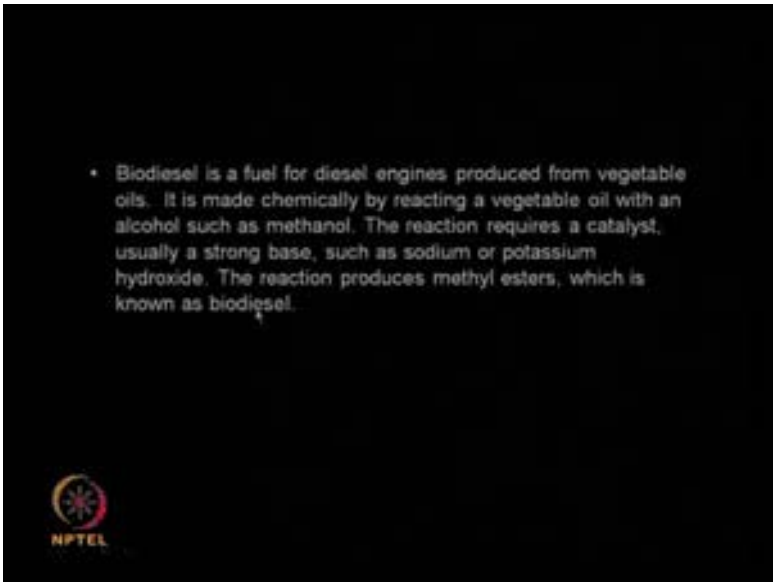

Now, let us look at another product Biodiesel. Biodiesel is a big thing now -a -days it is a fuel for diesel engines, it can be produced from vegetable oil. So, it is a green alternate to the oil which we get from the earth. So, it is a sustainable product, resource which is not

going to be consumed so; there is a lot of interest on biodiesel. And there are different types of vegetable oil which are being looked at for manufacture of biodiesel.

Even India is doing some research on retro ((Refer Time: 34: 33)) for manufacture of biodiesel. So, many countries all over the world are looking at vegetable oil, based diesel which can be used in cars or trucks even in aero planes as well. So, how is it made? It is chemically by reacting vegetable oil with an alcohol such as methanol.

So, basically it is a Transesterification reaction. So, the methanol is reacting with the vegetable oil that means, Trigliserida and producing your product. So, it requires a catalyst usually, a very strong based you use a sodium or potassium hydroxide. So, you are producing a methyl ester of the vegetable oil by producing methyl, ester you are reducing the viscosity of the vegetable oil. And you are improving its properties the physic so chemical proprieties and hence; it can be used as a mixture with normal petrol or diesel that is why it is called biodiesel, because it is made from vegetable oil.

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So, this is a Transesterification reaction. So, the methanol reacts with the trigliseride and then it produces a methyl ester that is; why it is called a transesterification reaction. So, the reactor will contain an alcohol that is; methanol, a catalyst and the vegetable oil. Generally, the reaction takes place within 1 hour and you use about 60 to 70 degrees temperature.

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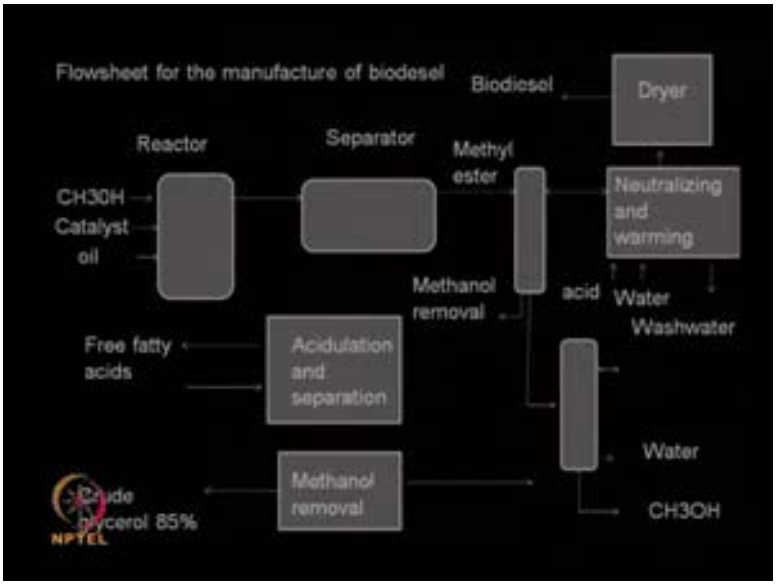

This is a typical flow sheet of a biodiesel manufacturing so this is called a reactor. This is a chemical reactor there is no a biological products coming into you are just adding an oil, catalyst which is a sodium or a potassium hydroxide. Then you are adding a methanol so it is not a fermenter or a bio catalytic reactor. It is a typical chemical reactor why am I giving this example; because there is a bio involved in the diesel manufacture it is a biodiesel that is why I am giving this example.

So, once you produce the biodiesel inside the reactor there is a separator because you are having a water, glycerol, oil, the biodiesel which is the methyl ester of the triglycerides. And then you are having some catalyst so many things are there present, inside. So, obviously you need to separate them and then you have several distillations columns. By using these distillation columns you are separating methanol, glycerol and biodiesel and then finally, the biodiesel is dried here. Dried means, the water in the biodiesel is completely removed.

So, this is how the reaction takes place so you have a transesterfication reaction. And then you have separators to remove solids, water, the glycerol, the oil and then finally, the biodiesel is dried here, which is used as a product, which can be mixed up with normal diesel glycerol is 1 of a large side product that is produced during the biodiesel manufacture. And people do not know what to do with this glycerol?

So, there are lots of researches being currently done on using glycerol to convert into

value added product. Because once everybody starts making bio diesel there is going to be so much of glycerol that is going to be produced and very cheap glycerol. And hence; the raw material cost will be low. If we can convert it into some value added product there is lot of scope.

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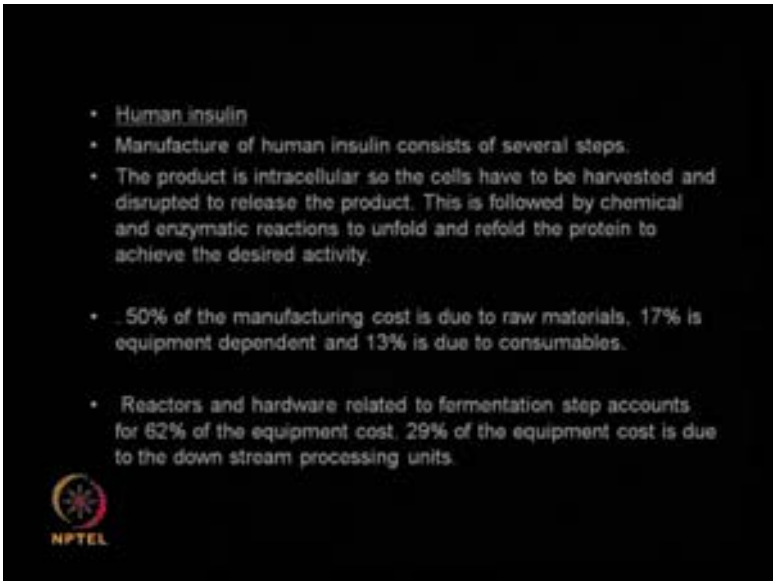

Now, next product we are going to talk about is human insulin it contain several steps because it is a product which is going to be used as a drug as a pharmaceutical product. The safety regulations are very stringent purity regulations is also very stringent. It produce intracellularly by the cells so in the cells have to be harvested, cells have to be broken down. So, that the product, that is; entrapped inside the cells get liberated.

So, there is something called the cell harvesting and there is something called the cell disruption. So, once you get the intracellular product, that is; not the insulin you need to convert it into insulin which is done through an enzymatic reaction. So, you need to unfold the protein and then again refold the protein, so; that it achieves the desired performance. Now, then it becomes the actual insulin. So, there are certain enzymatic reaction that takes place which unfolds as well as refolds the protein.

So, that the desired activity is obtained. 50 percent of the manufacturing cost is because of the raw materials, 70 per is because of the equipment and 13 percent because of the consumables. And 62 percent of the cost of the hardware is because of the fermentation and the reactors as well and 29 percent is due to downstream. So, you see about almost

30 percent of the equipments that are needed in the manufacturing of human insulin is downstream units. So, if I can reduce the number of units I am going to save on the equipment cost.

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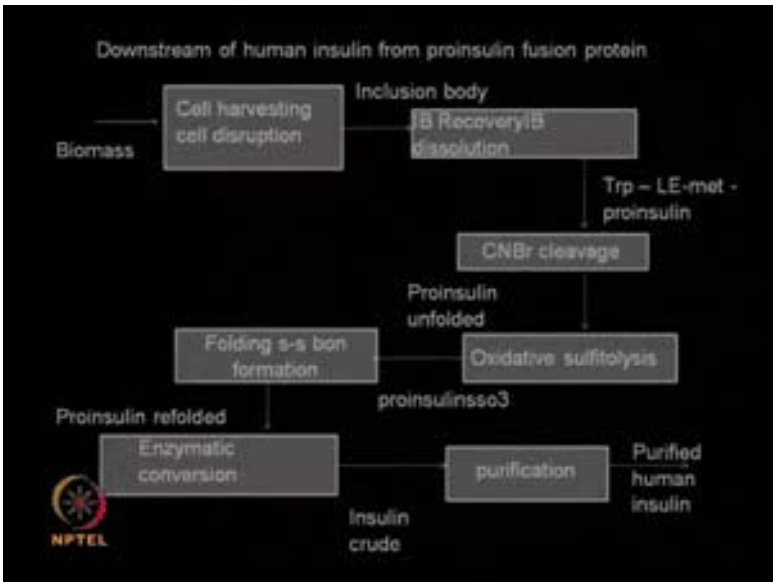

So, this is a typical flow sheet of human insulin starting from pro-insulin. So, what you do? You have fermentation you collect the biomass which is called cell harvesting. And then you break the cells how do we break the cells? We will talk about it in the future lectures. So, we break the cells, so; that the intracellular product comes out. And then you perform certain enzymatic reactions which folds, refolds your protein and make it very active and then finally, you purify your product, this is; what is called the purification step. So, all these are the downstream steps here. Once, you start collecting your cells.

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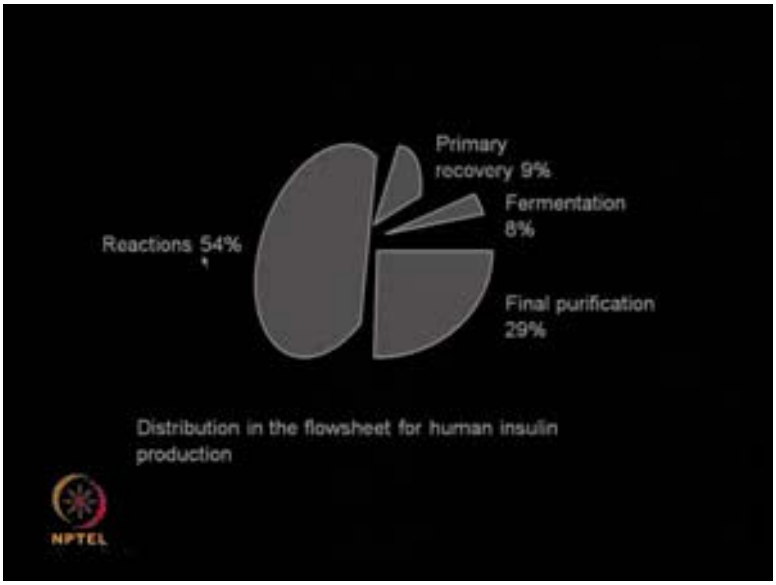

So, if you look at the break down of manufacturing cost for human insulin 50 percent is the raw material cost, 70 percent is because of the equipment, 13 percent is because of the consumables which are using in the various downstream steps what are consumables? Consumables may be solvents, may be gases which are using salts which used for a salting out so all these are called consumables. And 11 percent is because of the water which are using water disposal.

So, that means; effluent related cost so 50 percent of the cost is raw material related cost. So, there is lot of scope for performing optimization so that the raw material cost can be brought down. If you look at the various operations almost 50 percent related to reactions about 30 percent is because of purifications as I originally said this is a pharmaceutical product.

So, there are a lot of stringent regulations for it is purity and presence of nontoxic chemicals. So, you need to spend a lot of effort in purifying this product that is; why 30 percent of the cost is because of the purification step, 8 percent is because of fermentation, 9 percent is because of the primary recovering. So, the reactions which you know all the unfolding, refolding using enzymatic bio reactors contribute to 54 percent of the total production cost of this.

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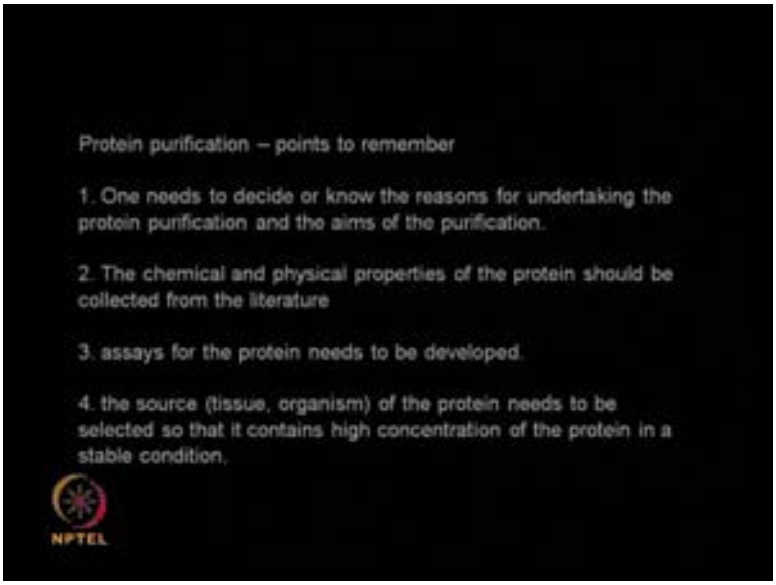

Now, I gave you examples, of several different products we started from alcohol synthesis, amino acids, streptomycin, and bio diesel and then finally, we looked at human insulin. So, each one has different types of downstream depending upon the product stability, depending upon the requirements of the product you have to resort to different purification steps. And I also showed what a contribution of each one of these is downstream in the overall manufacturing cost.

So, once you understand the various contributions of various units in the over total manufacturing cost. One can spend more effort more research and development efforts so that one can reduce the cost involved. So, it is very, very important to understand the effect of each of this downstream in the total manufacturing of the product as well as the selling price of the product.

Most of the biological products are proteins so when we are handling proteins we have to remember several points related to proteins unlike a normal chemical like an acetic acid or Butenoil or Butanediol or biodiesel. Proteins are biological molecules they lose their stability very fast depending upon the way we handle the ph condition, the temperature condition, the presence of toxic material.

So, we need to consider and keep several points in mind and that is what I am going to list out in the next 3 slides. So, if one is interested in purification of a protein you need to understand decide know the reasons for undertaking this protein purification, that means;

aims of the protein purification am I going to purify this protein for manufacturing or analytical purposes or characterization purposes.

So, depending upon the purpose for which I am doing this purification the approach or the methodology or the assays I am going to follow is going to be very different. So, you need to understand, what are the aims of this purification? What are the chemical physical properties is the protein? You need to collect it from literature what is the size of the protein? What is the molecular weight of the protein? How many strands it has? How many? What is the percentage of the b cash sheets? And so on.

So, you need to understand the chemical physical properties of these proteins, what are the assays for measuring the activity of the protein? So, I need to know those assays many of times it may be available in literature. Then I just need to bring it to my lab, do some modifications or optimize it. So, that it suits my conditions in some cases the assays might not be available that means; I need to develop my own assay to identify the activity of the protein in my lab.

So, that may involve considerable amount of time. Now, next step is source of the protein where am I going to get this protein from is it from a tissue, an organism which part? What will be the concentration of the protein? When I try to extract it from the source, will it be stable at those particular concentrated condition.

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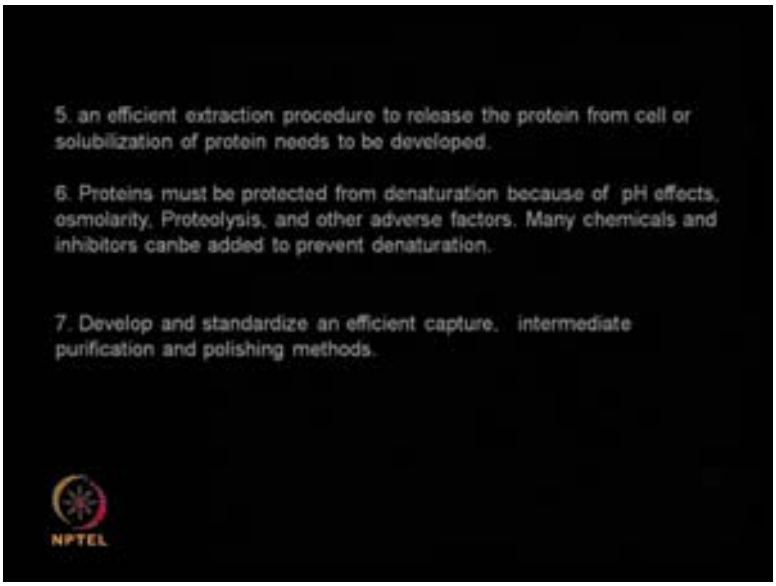

So, I need to understand that 5 point I should develop a good extraction procedure, for this protein am I going to follow a solvent extraction or a salting out type of approach or a page type of approach. So, I need to understand or decide on what will be my extraction procedure. Next is denatured of the protein as I said proteins get denatured very fast depending upon the ph, the osmolarity, temperature presence of other inhibitors presence of toxic chemicals.

So, do I have to protect my protein during the extraction procedure so that it does not get denatured. So, I need to keep those aspects into. I need to standardize the captured procedure, intermediate purification procedure and final the polishing and stabilizing procedure. So, I need to standardize all these things in my lab.

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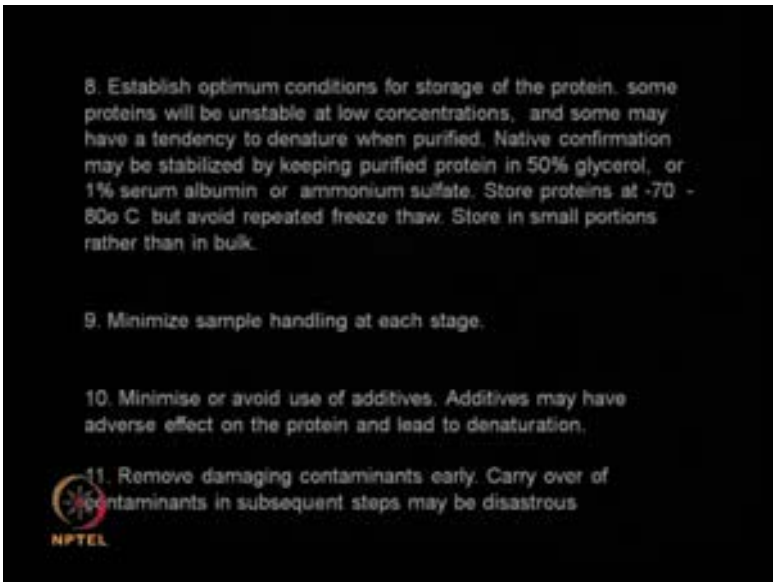

Next, is storage of the protein what are optimum conditions for storing my protein? Because some protein may be very unstable at low concentration, some needs very high concentration, some does not require should not have any water present in them. So, all these aspects need to be considered.

So, I may be like putting my protein 50 percent glycerol or some protein may be I would like to keep it in serum albumin sometimes you have to keep your proteins at minus70 or 80 degree centigrade sometimes you would like to store your protein in small quantities rather than storing the whole protein in a big bulk, that means; instead of storing my protein in 100 mili liter flask .I may like to store my protein in 500 micro liter flask so

you need to consider the quantity in which you would like to store.

Minimize sample handling at each stage. So, when I am doing intermediate purifications, intermediate assaying I would like to keep the handling very very small because as I keep handling the protein more and more many times. We are going to have a denaturation of the protein taking place. We have to minimize or avoid use of additives. Because additives may be toxic to the protein it may change the confirmation of the protein, the properties of the protein itself may change when I add the additives protein may get denatured over a period of time.

So, I should minimize or completely avoid the use of additives. And if I have to add additives I should know whether the additive is going to affect the protein activity over a period of time. And later on if I have contaminants present in my protein mixture it is better to remove the contaminants as early as possible rather than keeping the contaminants during the intermediate stages.

Because if when I am purifying a protein the contaminants itself may get purified or concentrated then they may start acting on the protein. And they may denature the protein. So, you need to remove the contaminants as early as possible and one need to look at how early we can remove the contaminants so that they do not get accumulated over a period of time. So, these important 11 steps need to be resorted to if one is handling a protein type of bio molecule, unlike handling simple chemicals like acetic acid or biodiesel as I said earlier.