**Principles of downstream techniques in Bioprocess – a short course**
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**Lecture – 01**
**Introduction**

Hello everyone. Am going to talk about the principles of downstream techniques, in bioprocess. This is going to be a short course, 20 hours lecture and this is a short course, which you can make use of, with respect to a long course which I have in the nptel, already loaded couple of years back. So in the next 20 lectures, I am going to cover as many downstream techniques as possible. I will give a few worked out examples as well as I will cover some of the principles involved in downstream.

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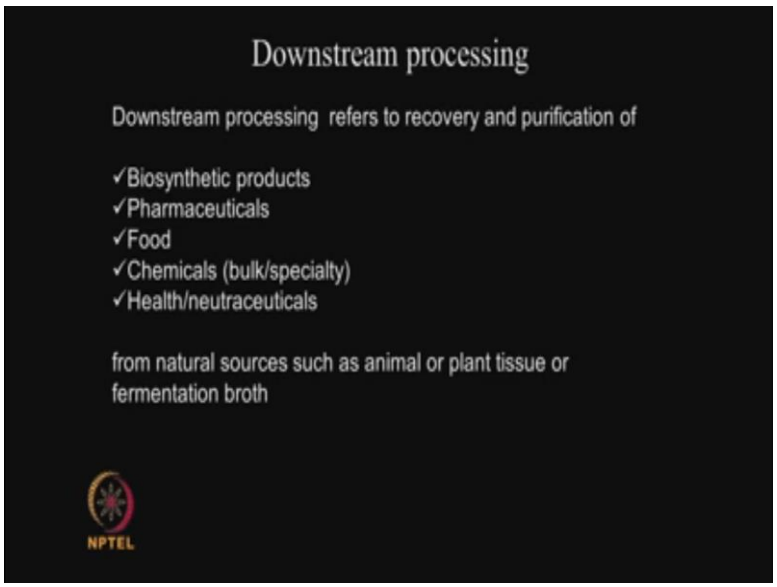


The first lecture is on introduction, so what does downstream means? Downstream is a process where we try to recover, purify the products that are coming out, It could be metabolites, it could be enzymes, it could be a biomolecule that is coming out of a fermenter or a bioreactor so it could be a pharmaceutical product, it could be a food grade chemical, it could be a bulk chemical, it could be a speciality healthcare products or neutraceuticals.

So a large number of products which are manufactured using a fermentation root or a bio transformation root is what is meant by downstream. So the product could be manufactured from natural sources or it could be from a plant, animal bacteria, fungi and so on actually from the fermentation process and that is what it is all about we are going to talk about actually.

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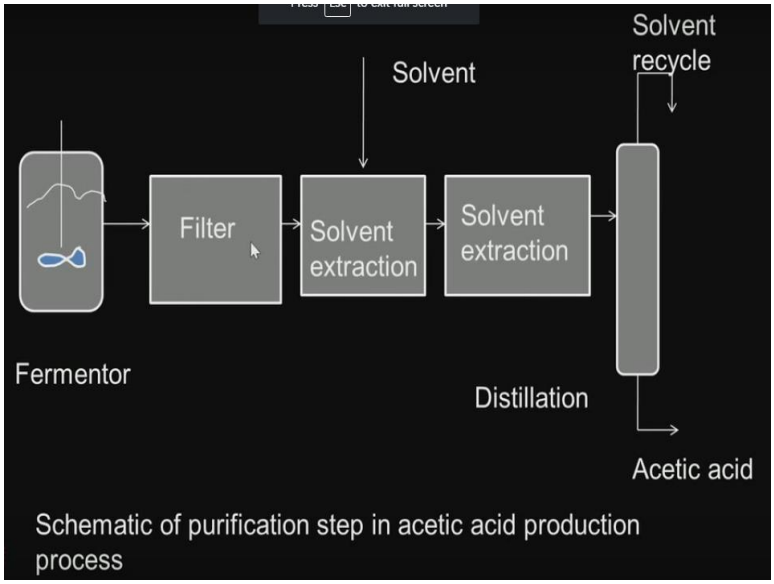


So this is the typical downstream process for the purification of acetic acid. Okay. Acetic acid is produced by a fermentation process here especially the vinegar, which you call is nothing but acetic acid and it is produced through the fermentation rule. So immediately after the fermenter you have a filter. Why do you need a filter? You need a filter for removal of the dead biomass and salts and other undissolved products and then it goes into a solvent extraction were you use a solvent to recover your product.

And then in the distillation column, you remove the solvent and also the acetic acid. So acetic acid comes at the bottom of the distillation column and the solvent comes at the top and solvent is recycled back into the extractor. So this is the typical flow sheet of a downstream process. You will have a filter, you have a solvent extractor and you have a distillation column. So this is a very simple downstream process.

Sometimes you have very complicated process involving chromatographies and crystallizers and so on actually. So in this particular case we have just 3 different units of downstream. One is the filter, other is the extractor, third one is the distillation. Please note that here the acetic acid is not a thermally labile product, so you do not mind using a distillation column for removal of the solvent in case acetic acid or any metabolite is thermally labile, then you have to be very careful. You cannot use high temperature.

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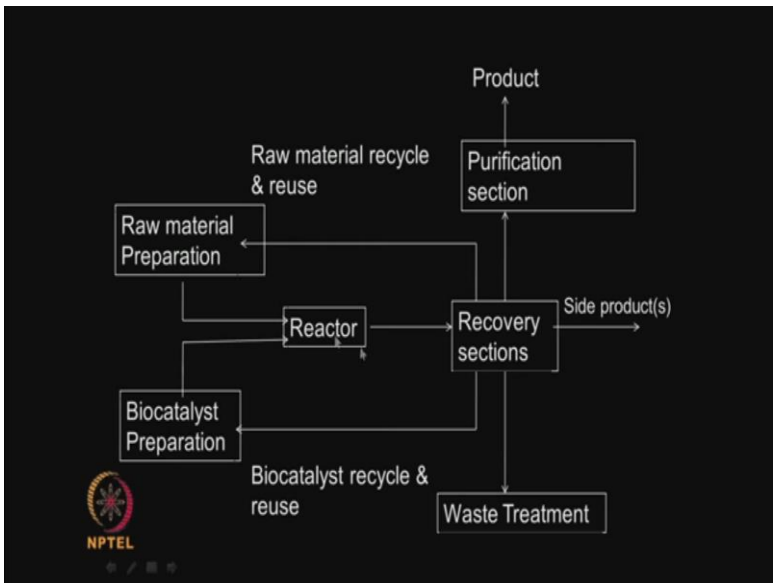


So a typical bioreactor, a typical fermenter will contain so many different operations. This is the heart where you have the reactor or were you have the fermenter and here on the left hand side as you can see here, okay, you are preparing your raw materials. You are preparing your catalyst or microbe, could be a bacteria or a fungi and so on... And these steps are called the upstream processes whereas on the right hand side, where after the fermentation, you have the recovery; you have the purification of your product that is called downstream.

Okay, so you have the recovery of the product, sometimes you may also end up having side products and finally you are purifying your product here, depending upon the type of nature of the product, if it is a pharmaceutical product you may have to resort very high end purification product. Whereas if it is bulk chemical, you need not resort very high end purification so you could have several purification steps here.

If it is a pharmaceutical product so when you are producing a product using a fermenter you may end up having side products as well as you may also have wasteful products which goes into the waste treatment plant actually. So after the recovery, sometimes the raw materials are recycled, sometimes the reaction is not complete, not 100%. So you would like to recover the raw materials, the salts and recycle back and at the same time, if it is a bioreactor, biotransformation, you may recover your biocatalyst and recycle back that is why you have 2 recycle steps.

So whatever you have here is called the downstream and whatever you have here is called the upstream and our focus is going to be on the this side of it and we will look at different types of downstream for removal of solid, liquid, for extracting a liquid from another liquid, for drying a solid and for purifying a solid and so on actually okay.

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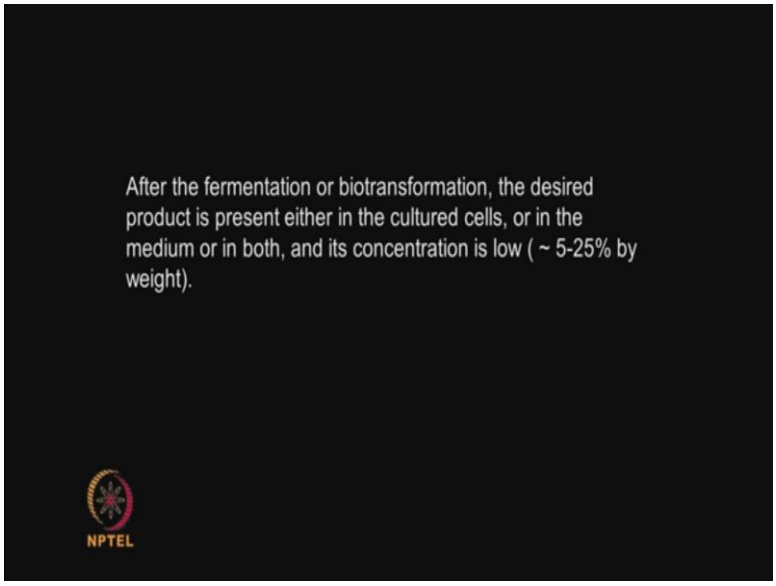


So after the fermentation or biotransformation, the product that is the desired product is present either inside the cells or in the medium. So sometimes your product is inside the cells that is called intracellular product. Sometimes the product is in the liquid medium that is called the extracellular. Okay. So you would like to consider both, whether it is intracellular or extracellular and that is what you are trying to recover.

Generally a concentration after a fermenter is very very low, hardly 10, 12 % for example if you take ethanol fermentation you do not you may just cross 15 % with the current genetically modified yeast. So the amount of ethanol after fermentation may be less than 15% and finally you would like to recover and make it very pure going right up to 99 %. So that is where the downstream process steps come into picture.

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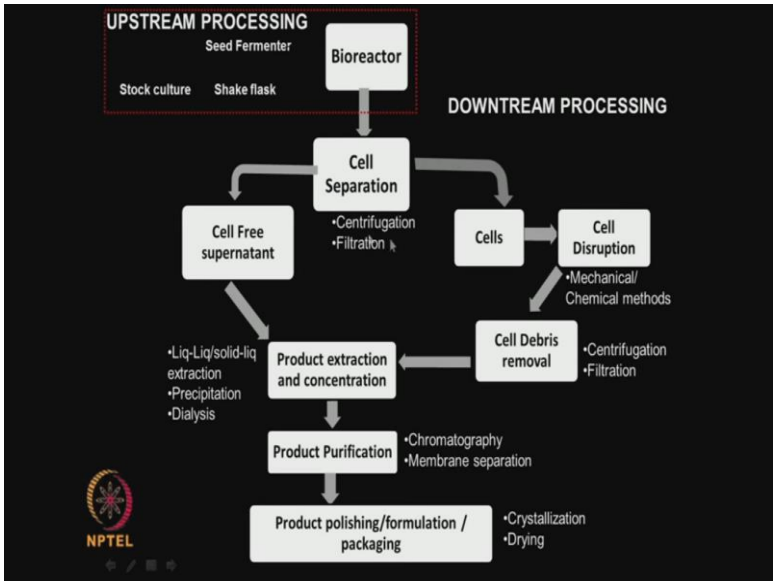


So the various processes, various principles of downstream comes into picture. So as you can see

the very first downstream is going to be a cell separation or centrifugation or a filtration and so on you are removing a solid, from the liquid. The solid could be a live biomass, a dead biomass, intracellular products or it could be salts, undissolved salts, metabolites. So the very first step is the cell separation and depending upon whether your product is intracellular

Or it is extracellular what you do is, if it is an intracellular, you are interested in the cells, so what you do is, you collect the cells, disrupt the cells and then remove the debris using again centrifugation and go into product recovery. If you interested in the extracellular product, what you do you filter the cells and throw them out so you take the cell free supernatant and then go into product recovery. So these are the 2 parts by which the downstream differs.

If it is an intracellular product, you are interested in the cells, not in the liquid, if you are extracellular products, if you are interested in the liquid side of it and not the cells. So once you have taken the product, you have to concentrate the product, then you have to purify the product, so this is called the product extraction and concentration or enrichment. Here you may use different types of downstream like liquid-liquid extraction, precipitation, dialysis, different types of membrane processes and so on and finally you go into the product purification.

Product purification is where you are trying to bring the product from, may be about 40 50 % right up to 90 or 99 plus, depending upon the type of product. So you may use different types of chromatography you may go into membrane separations and so on actually. Okay that is where you have the product purification and finally you go into product polishing, formulation, packaging, so you may add some agents which will prevent the product denaturation, stabilize that, they are called stabilizers or the compound which prevent the denaturation.

Then you will do the crystallization if you want the solid product and then finally you may also go into drying depending upon whether the product is solid or not. So these steps of crystallization or drying are carried out if your product is a solid otherwise you may stop with the liquid product but generally you do add some sort of a stabilizer so that you prevent the product degradation you prevent formation of bacterial growth and so on actually.

So these are the various downstream you have, the centrifugation, filtration, you have the liquid-liquid extraction, precipitation, dialysis, chromatography, membrane separation, crystallization and drying and if it is an intracellular product you have cell lysis or cell disruption again you have centrifugation and filtration.

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So the upstream is where you are preparing the growth media, you are sterilizing the growth media and you are preparing the inoculum or the bio-catalyst that is upstream. Downstream is rest of the various unique operations which I talked about actually.

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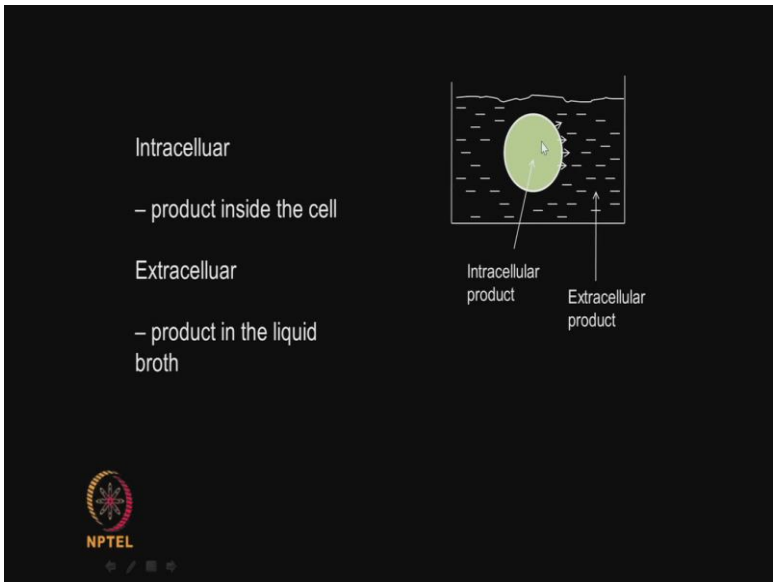


So if it is an intracellular as I said, the product will be inside the cells, if it is an extracellular the products will be outside, so inside the cells it could be in the form of Golgi body. It could be between the cell membrane and so on. So intracellular requires some more extra operations which could be mechanical, which could be chemical, which could be thermal and so on actually. So extracellular products is generally more expensive than the sorry extracellular products is generally cheaper than the intracellular products.

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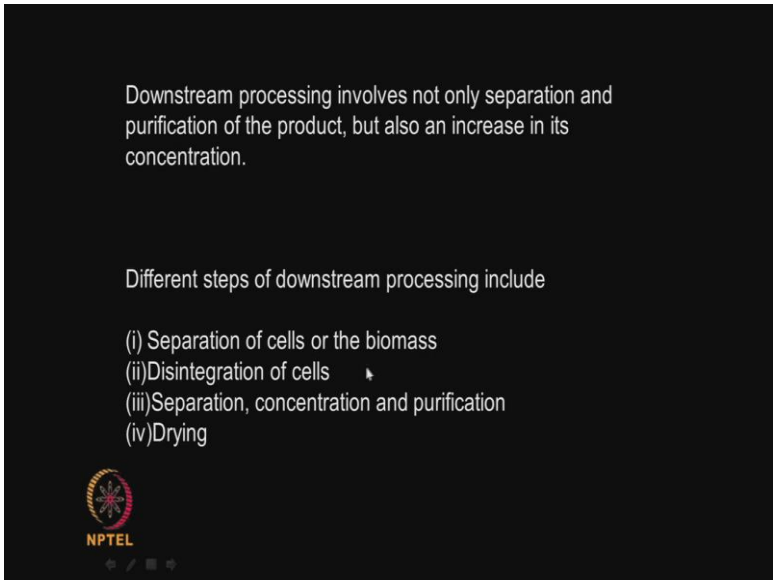


So basically the downstream steps involves separation of cells, cell debris and biomass then disintegration of the cells, then separating the solid and the liquid then concentrating your product and then purifying your product and then drying. You can broadly group the various downstream into 4 large categories okay.

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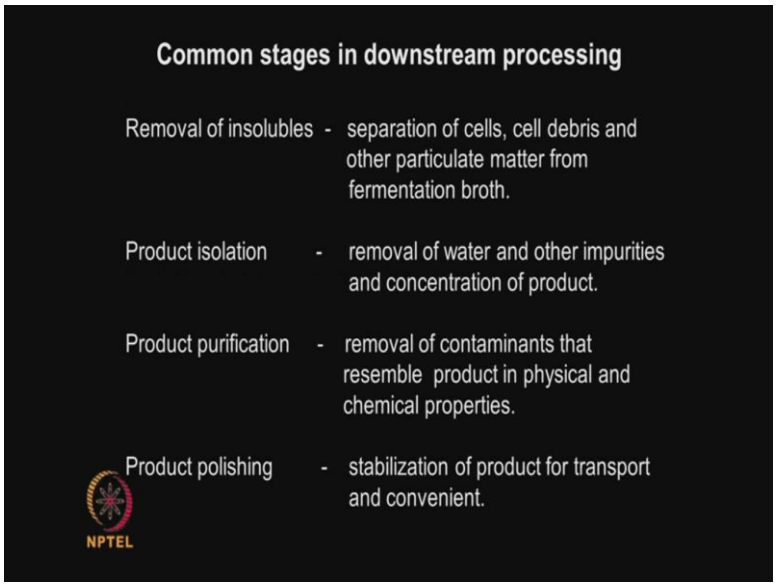


So removal of insolubles, so what you do you are separating cells cell debris, particulate, matter salts, fermentation, broth and so on. Then product isolation, so here you may be removing water impurities, concentrating your product and then later on you are doing the product purification, where you are removing contaminants and finally purifying the product to very large purity and then polishing, where you are trying to keep the product ready for transportation and convenience. Okay.

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So removal of insolubles involves filtration, centrifugation, microfiltration, whereas isolation of the product will involve cell disruption, extraction, adsorption. Purification will involve chromatography like affinity chromatography, size exclusion chromatography, reversed phase chromatography. Then product polishing will involve crystallization, lyophilisation, desiccation, spray drying, adding chemicals or agents which will prevent the disintegration of your product and so on actually. Okay.

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So let us look at very interesting problem here. Okay. Suppose there is a bioreactor or there is a fermenter where the yield in the reactor is 95% that is 0.95. Okay. You take the fermentation broth and you have one separator and efficiency of the separator is 98% that is 0.98. So what will be the overall product yield do you think? So yield in the reactor is 95%, the efficiency of the separation is 98%. So what will be the overall product yield?

So what you do you multiply 0.95 and 0.98 okay, so that comes through 93% that means the overall yield of one reactor or a fermenter with one separator, each having 95 and 98% yield and efficiency we end up with 93% overall product yield. Okay. That means we have lost 7% of the product amount instead of getting a 100%, we are losing 7 %. Did you see that? Very interesting. Although the 95 and 98 looks very very large but overall our product yield is only 93%.

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Okay so look at another problem, as I said any process will have several downstreams. Filters, chromatographies, extraction and so on. So imagine a process where you have a bioreactor or a fermenter, 95% yield and we have three downstream units here, each one is 98, 98, 98. So what will be the overall product yield? Okay now you know how to do that right? So you just have to multiply all of them 0.95*0.98*0.98*0.98. So if you do that, what happens?

You end up in 89% that means overall product yield is only 89%. At the end of these three downstream steps, we are getting only 89% of our product that means almost 11% of the product is lost. Okay. Because of the separation, because of the poor yield in the reactor, so although it looks very large the 95%, 98% looks very large but when you consider all of the together you lose little material in each one of the downstream.

So when you multiply all of them, you ended up with a number called 89% which is a much lower. That means you are losing almost 11% of your product in a particular flow sheet which contains one reactor and three downstreams.

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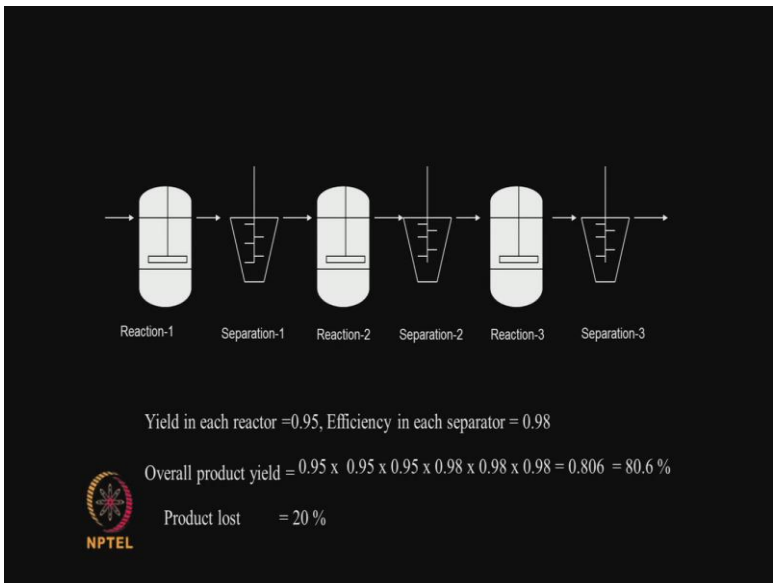


Now let us like to make it more complicated you have three reactors or three fermenters and you have three downstream units like this. Okay. The yield in each of the reactor is 0.95, 0.95, 0.95 and each of the downstream is 0.98, 0.98, 0.98, so if you multiply all of them to get the overall product yield, what you get?

You get 80% only, that means 20% of your product is not getting recovered at all in your downstream, so what does these three examples show you? Although the efficiency of separation looks very very high, we think 98% is a very large number but when we have many downstream steps with the 98% yield and when you multiply all of them to get the overall yield. Our overall yield comes down quite a lot.

So as you can see in this particular example, the overall yield has come down to only 80% that means 20% of our product is lost somewhere, so what it means is that, our downstream steps have to very very efficient. We cannot afford to lose any material in the downstream because when you have many downstream steps and when you calculate the overall yield and we try to multiply each of the yield together, we end up with a much smaller number which looks very very poor.

So although it looks 98% in each of these downstream steps, finally we end up with 80% product yield that means 20% of the product is lost somewhere. That is very large especially if it is a pharmaceutical product or if it is a product which requires very high purity then we are in real trouble. We cannot afford to lose so much of these materials. So when we design downstream steps, we have to be very very careful, as the efficiency of the separation has to be extremely high, even 95, 98% if you think is sufficient, definitely it is not sufficient.

You may have to go and try to improve it further and further. That is very very important lesson

which you have learnt from these three slides okay.

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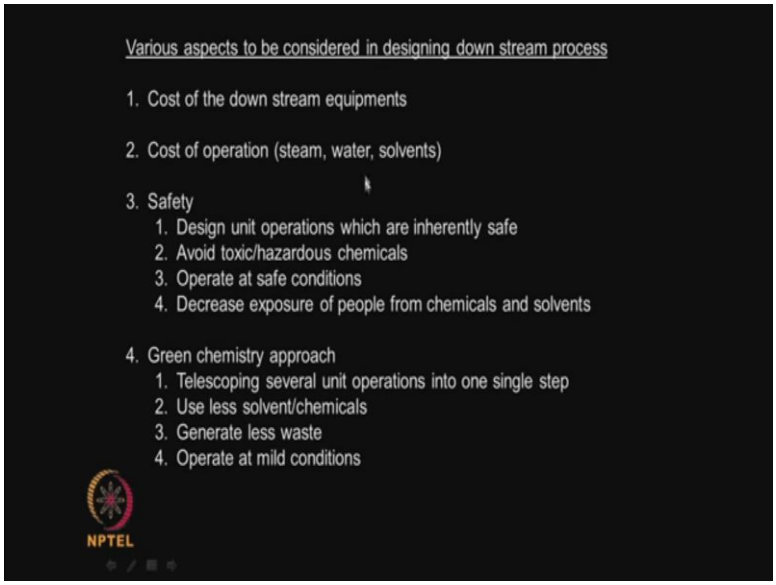


Okay, so other aspects which we need to consider when we are designing a downstream process, cost of the downstream equipment, so how costly is it. Is this chromatography very expensive? Is a filter very expensive? Is a centrifuge very expensive? Should I go for a filter which is cheaper than a centrifuge? So you need to consider all these aspects when you are designing a downstream. Sorry cost of operation, does it require water? Does it require solvent? Does it require steam?

Does it require hot oil? Because each one of them will have its own cost. Can I do things with the steam or hot water rather than going into hot oil, which may be expensive. Can I recycle this hot water? Can I recycle this hot solvent and so on we need to consider the cost of operation. Next is safety. What are the safety issues I need to consider? Okay. Because it is going to be handled by human operators, so am I using toxic chemicals? Am I using unsafe conditions?

Am I using very high pressures? Am I exposing the person with very high solvent concentration. Then finally I need to consider, can I combine two downstream steps as I showed you in previous example? If I have three downstream, if each one is 0.98 efficiency and if I multiply all three of them together, I may end up with much smaller number, so can I combine two downstream into one? Can I use less solvent or chemicals? Can I generate less waste?

Can I operate at milder conditions rather than very high pressure and high temperature or very cold conditions? Can I operate at ambient conditions? So these approaches should also be considered when I am designing a downstream processing step

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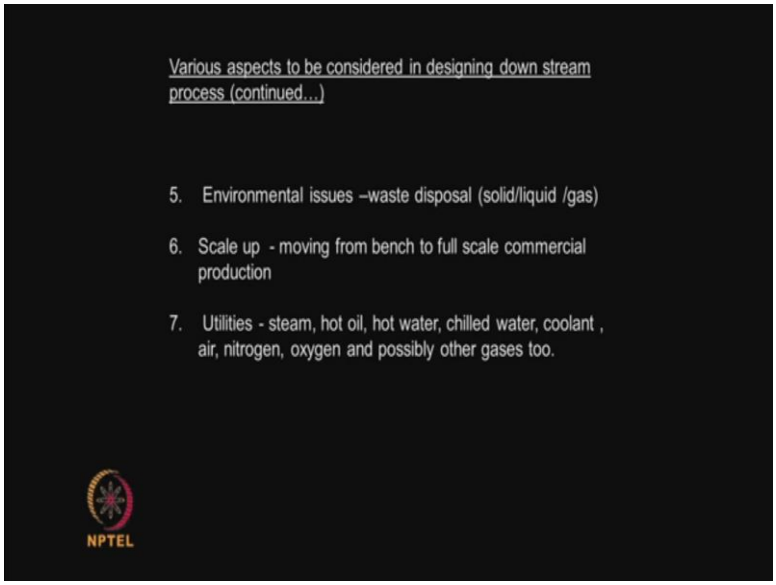


Then what are the environmental issues? How much waste am I generating? Am I generating lot of solid waste? Am I generating liquid waste especially in liquid-liquid extraction? Am I going to have lot of solvents being used so am I recovering all those solvents because I cannot afford to throw the solvent out. One is the cost of the solvent could be very high and number two is I will be generating waste solvent which cannot be thrown out like that if I am using two different solvents.

Then I am in big trouble because I will be mixing those solvents and then recovering each of the solvents independently or individually is going to be very difficult. So try to use less number of solvents instead of mixing solvents. Gas.. Can I just vent the gas out? Suppose I am producing carbon monoxide or carbon dioxide in the fermenter or in the downstream, can I just vent it? That is not allowed. Okay. Then next question is scale up.

We all develop a processes fermentation processes in small shape flask 100 ml quantity but finally this process has to go into a manufacturing, which may be running into meter cubes volume that means thousand of litres, so how am I going to scale up this. A downstream how am I going to move from a small millilitre scale right up to metre cube scale thousands of litres of extractor thousands of litres of chromatographic column so I need to consider those aspects.

And finally the utilities how much steam am I going to use? Am I going to use hot water, hot oil, chilled water, cooling am I using going to use different gases, air, nitrogen, oxygen and carbon monoxide and so on. So all these needs to be considered when I am designing downstream. So a downstream which will use less of these will always be preferred compared to a downstream which will be using lot of these utilities and environmental issues like a waste disposal and so on actually.
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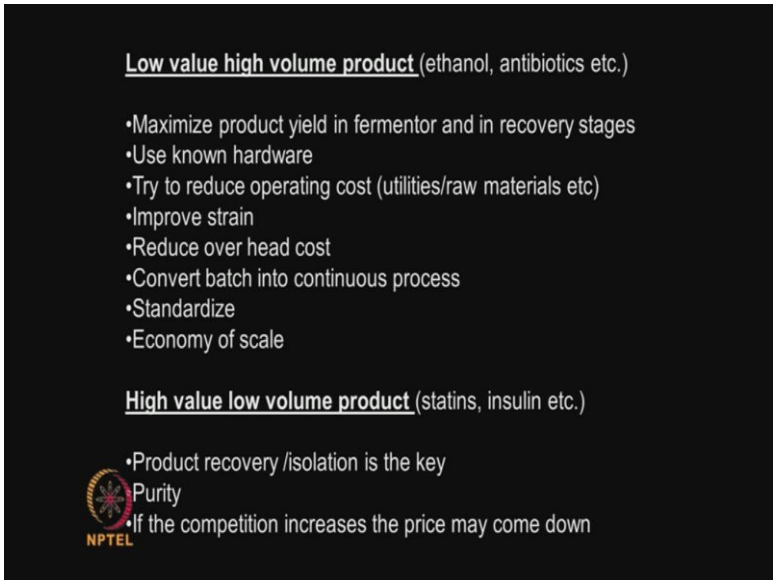


Okay there is something called low value high volume product and high value low volume product For example if you take some pharmaceutical product like insulin, statins, certain drugs it may be anticancer drugs for example, it will have very high volume. For example it may be costing lakhs of rupees per kg of a drug, so it is generally manufactured in very low volume. So annual production could be only in kgs which will not be in 100s of tons but it will be in 100s of kgs.

At the same time you will have something called low value high volume product. For example acetic acid, cost is very low but then you will have to produce in large quantities. Same thing with the butane diol, cost is low but you may have to produce large quantities. Some of the antibiotics, the cost is low but you may have to produce large quantities ethanol. So these are the examples of low value, they will be running into rupees but it has to be produced in very large quantities.

Okay. So they are called low value high volume products and the other one is called the high volume low value product. So when you are designing low value high volume product, you want to maximize the yield and you want to maximize the recovery because as much material as possible you must get, you must use know hardware do not think of designing new downstream. Use the already available from vendor hardware you must use low amount of raw materials utilities. Okay.

Do not use too much of raw materials and utilities and increase the operating cost. You must try to improve the strain. So lot of genetic engineering modification of the strain should be done, so that the strain keeps producing more and more. For example, ethanol, for the past 20 years or 30 years there are almost three or four different types of strain modifications that have been done, so that the current strains can withstand much higher concentration of ethanol in the product reduce overhead cost.

Okay. We will talk about overhead later on in the course but you must try to reduce the overhead cost that means cost involved in manufacturing to convert batch into continuous process.

Can I make ethanol continuously? Can I make bio-ethanol continuously rather than in a batch? So because if I do it in batch, I need to produce the product, I need to remove the product again, charge the raw materials so you are losing lot of time, so we have continuous reactor. Can I have continuous downstream processes standardize everything and then okay finally economy of scale normally low value product you need to manufacture in very large quantities okay that way the scale of operations reduces your overhead cost in that way makes your product cheaper.

So generally low value product are manufactured in thousands of tons, ten thousands of tons whereas on the contrary high value low volume products like statins and insulins, product recovery, isolation in the queue is the key because you do not want to lose even a small gram of product. Because even that gram of product could cost you thousands of rupees because the value is very high.

So you want to have very good downstream processes so that you recover as much as possible. So you do not have to mind putting extra chromatography, extra extractor because the product recovery has to be complete, total 100% product recovery, purity is very important as you know pharmaceutical products, you cannot afford to have very impure product you would like to keep very high purity product.

So what happens the combination increases suddenly, the prices will come down and then a high value product can become a low value product. Okay. So initially you may be the only manufacturer of this so your cost selling price of the product is very very high but then after sometime the product is becoming cheaper because there are lot of competitors coming into market so you have to be moving from this strategy into this strategy.

As you can see both the strategies are very different for the low value, you have some strategy and for the high value you have different strategy. So initially when you are the only manufacturer, you can sell it at any price but after sometime when there are many competitors, the product selling price has to come down so you have to change your strategy in the manufacturing in the downstream in the strain and so on actually okay.

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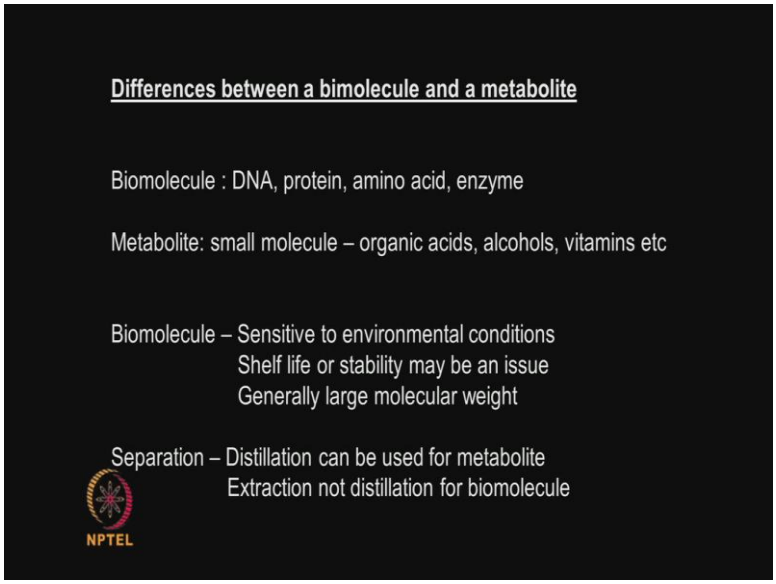


I also would like to talk about biomolecule and the metabolite. Biomolecule could be a DNA protein, amino acid, enzyme, whereas a metabolite could be that is produced by the organism it could be a secondary metabolite it could be a organic acids, alcohols, vitamins and so on. So generally a biomolecule may have a higher molecular rate as a metabolite may have a lower molecular rate, so the biomolecule will be sensitive to environmental conditions like pH, temperature, shelf life and so on, whereas a metabolite might not be so sensitive to environmental conditions as well as the storage conditions.

So metabolites we can use the distillation to separate like ethanol, acetic acid, we can use distillation to separate whereas if I am having a biomolecule, they are very temperature sensitive, very labile. So we have to resort to extraction not distillation. So that is the difference between biomolecule and a metabolite.

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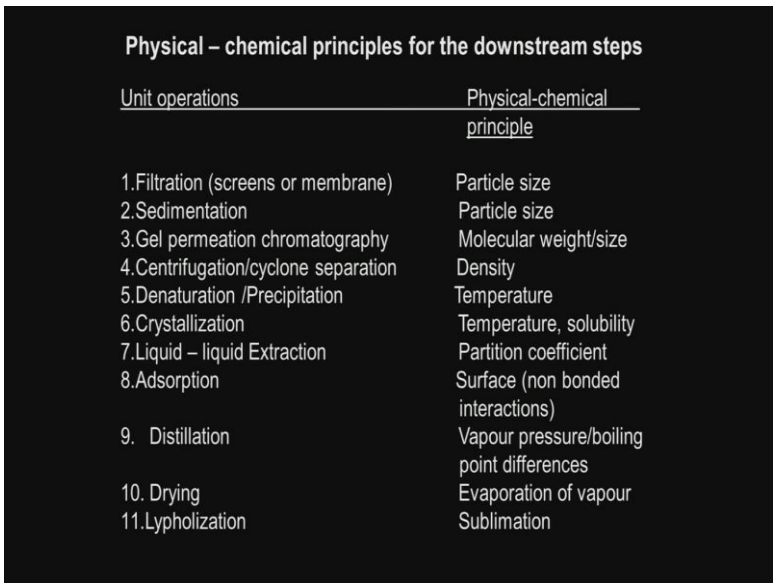


So there are many principles by which each of the downstream step works and the next two slides show them. For example, filtration, if you take it works on particle size so larger particles are stored on top of the separating filter clock smaller particles go. Sedimentation again is based on particle size, larger particles settles down. Chromatography, for example, gel permeated chromatography works on molecular weight or size difference.

So in different sizes, different times we are going to spend some time on that later so different sizes of molecules biomolecules come at different times hence the separation. Centrifugation, cyclone again is based on density precipitation and denaturation is based on temperature. Crystallization is based on temperature, solubility. Liquid-liquid extraction is based on partition of the solute between two liquids and a solvent adsorption is based on surface phenomenon where certain solutes are adsorbed on solids, based on non bonded interactions.

Distillation is based on vapour pressure and boiling point difference. Drying is based on evaporation of vapour. Lyophilisation is based on sublimation where a liquid completely changes into vapour and comes out liquid could be water or any solvent.

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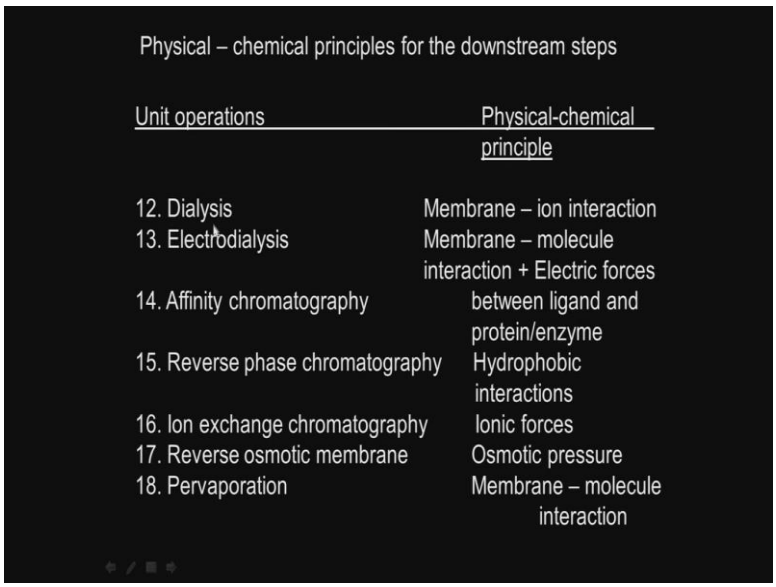


Dialysis is based on membrane interaction, Electrodialysis is based on again membrane processes. Affinity chromatography is between ligand and a protein or a enzyme. Reverse phase chromatography is because of the hydrophobic interaction. Ion exchange chromatography is of ion, ionic forces. Reverse osmotic membrane is based on osmotic pressure pervaporation is based on membrane molecular interaction.

So you see so many unit operation and downstream and each one of is based on certain physical or chemical principle and the principle could be because of the vapour pressure changes solvent partition. It could be because of adsorption and ionic forces. It could be because of particle sizes or molecular weight differences because of the hydrophobic nature. It could be because of the ionic forces and so on. And we are going to cover some of this over a period of next 20 lectures and I hope you enjoy subsequent lectures and if you have any doubts you can always email to me.

Thank you very much.