Course on Industrial Biotechnology
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Lecture 44
Module 9
Baker's Yeast Fermentation (Continued)

Welcome back to the course on Industrial Biotechnology I was discussing about the Bakers yeast fermentation process so I am going to continue that in the last lecture I tried to tell that Bakers yeast actually basically we call it as a food yeast and this is produced by using saccharomyces cereviciae now this is used for leavening, leavening of the bread and I told you that it is a good source of nitrogen also vitamin content, so and now I am going to discuss this process in details.

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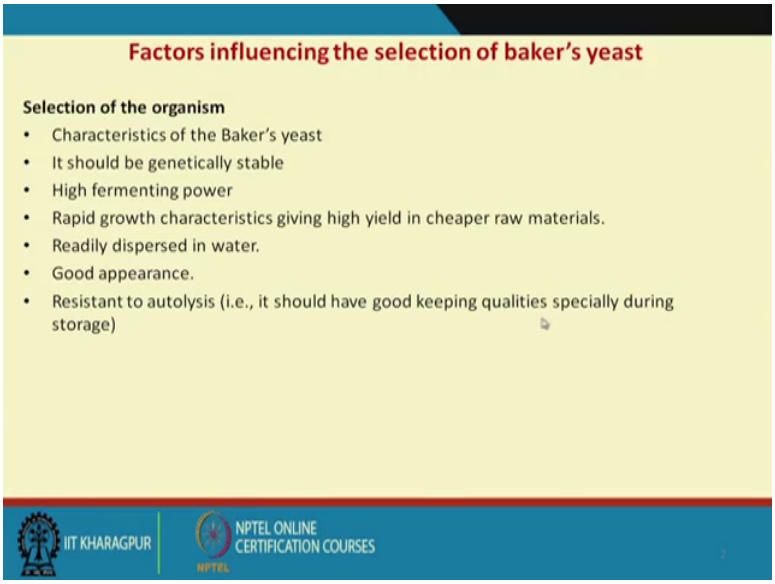

The most important thing that we have the factors that affects the Bakers yeast fermentation process I told you the selection of the microorganism plays very important role now question comes what should be the characteristics of the microorganism first thing that it should be genetically stable which is very important because if organisms are genetically stable then and only then the biochemical characteristics of the organism will be same so and it should have high fermenting power so that degradation of the substrate will be very fast and then if the rapid growth characteristics giving the high yield on cheaper raw materials because the raw materials more cheaper your cost of production depends on that, if the raw material is cheaper your cost of the cost of the yeast cell will be less.

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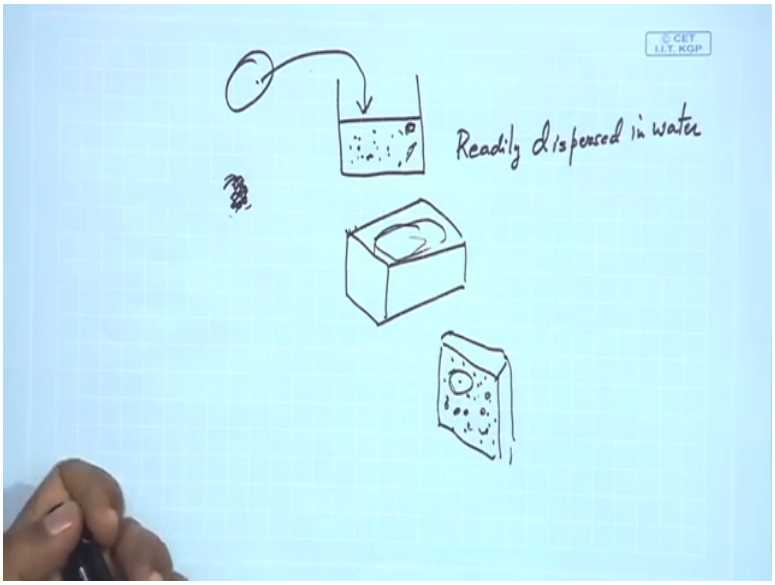

And then readily disperse in water because this let me explain is very well because because this is a special characteristics we want for the yeast cells. Readily disperse in water now what does it mean suppose we have yeast cells here and we make a cell suspension here now if cells are there like this.

Suppose some cluster of cells are like this and cluster of cell means lot of cells they are binding together like this now I told you this is used for the bread making purpose now when you prepare the dough suppose you are preparing this is a container and in this container you keep the dough, the the the dough you prepare and keep it now if your if a yeast turn or disperse properly, if they are not segregated then and they form this kind of cluster then what will happen.

When when you you when yeast when your dough bulge and dry it and then when you cut the bread if you take the surface of the bread if you this is the this the slice of the bread if you see it then you will find there some big holes is there, some small holes are there because why the big holes are there due to the presence of the cluster of cells and due to the presence of the cluster cells they produce more carbon-di-oxide and they make a big hole here and since this here we have seen single yeast cell it it form the smaller holes.

So they producity will be different so this is undesirable and that is why one important characteristics of the yeast cells is the readily dispersed whenever you prepare a suspension it should be uniformly dispersed in water. Then good appearance because whenever we go to the market and purchase any kind of product the appearance of the product also very

important factor so from the appearance we have to see that whether it is good and bad. You can usually yeast should look like perfect white colour but if it is it is of different colour that means may be maybe due to some contamination problem.

And resistance to autolysis I mentioned that also this is you should not degrade, if degraded it then you will not get the rapid this fermentation characteristics. It should have good keeping qualities specially during storage.

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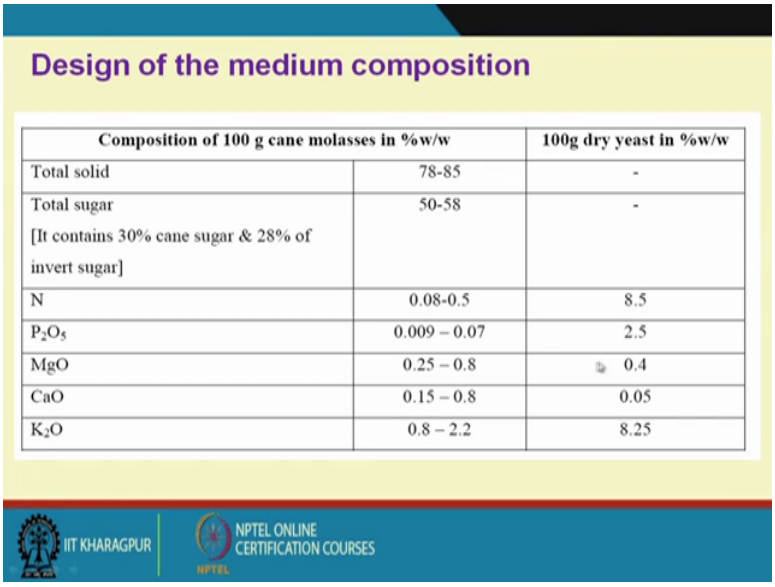

Now now another thing very important thing that we have that is the media because after when you when you have good microorganism now good media also very important now one very important thing that we have in the mind how you design the composition of the media particularly I have taken this Bakers yeast fermentation as the kind of example.

That how you can is it possible what is the what is the methodology through which we can design that what are the component required for this fermentation process. Now we already observed that molasses is the best raw material for the yeast fermentation process why the reason is that it contains good amount of sugar, good amount of vitamins and good amount of nitrogen source, minerals and other things which is required for the growth of the organism.

So we do not have any problem, the growth of the organism is not a problem at all in this but question comes that whatever whatever material whatever component present in the cane molasses whether is is it is suitable for yeast to growth or not that we shall have to find out that is exactly because initially when I talk about the growth characteristics and all these

things then I try to tell you that when you when any microorganism grow they require couple of things.

One is nitrogen source, they require proper carbon source they require minerals, they require vitamins and everything has different purpose I told you carbon is used for the cell mass formation. Carbon is used for the energy and also carbon sometimes used carbon is used for product formation if the product is other than cell mass. Nitrogen mostly contribute for the cell mass formation and minerals and vitamins they only contribute as a cofactor in the metabolic pathway for the different enzymes.

Now here that if you look at the composition of the cane molasses I have taken the example of cane molasses because India we have mostly the cane molasses availability because we have lot of cane production and mostly the sugar industry based on the cane so cane molasses is our major raw materials available in India and if you look at the composition is like this, total solid the sugar content is 50 to 58 percent, nitrogen 0.08 to 0.5 percent and phosphorus has P2 O5 0.009 to 0.07percent.

Magnesium oxide 0.25 to 0.8 percent calcium oxide 0.15 to 0.8 percent and K2O what is that is 0.8 to 2.8 to 2.2 percent but if you look at because because yeast has to grow so what we do that we we take the dry yeast and try to find out what is the the inimitable analysis of this yeast cells and we find the yeast cell contains about 8.5 percent nitrogen source phosphorous is 2.5 percent on dry basis then magnesium oxide is 0.4 percent and calcium oxide 0.05 percent and K2O 8.25 percent.

Now we know that yeast cells might be containing when it present in the fermentation medium can containing more than 90 percent of moisture so so naturally the concentration will be little bit less than that and the weight condition and and and and now one thing apparently it looks that two things is quite less in the in the cane molasses one is nitrogen contents if you consider the nitrogen content in the yeast cells and nitrogen content in the cane molasses quite less and and phosphorous contains also significantly less as compared to other things is more or less same that we have as compared to this but sometimes we find the magnesium oxide also little bit late as compared to what is required for what is have been the yeast cells.

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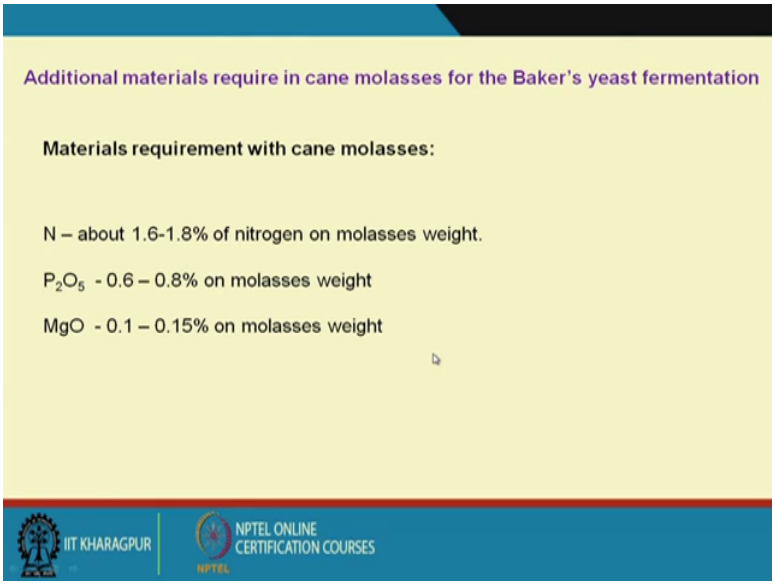

So it has been observed that additional material required for the molasses fermentation is like this nitrogen 1.6 to 1.8 percent of nitrogen on molasses weight, Phosphorous P2O5 as 0.6 to 0.8 percent on molasses weight, MgO 0.1 to 0.15 percent on molasses weight. So this is the requirement we have calculated.

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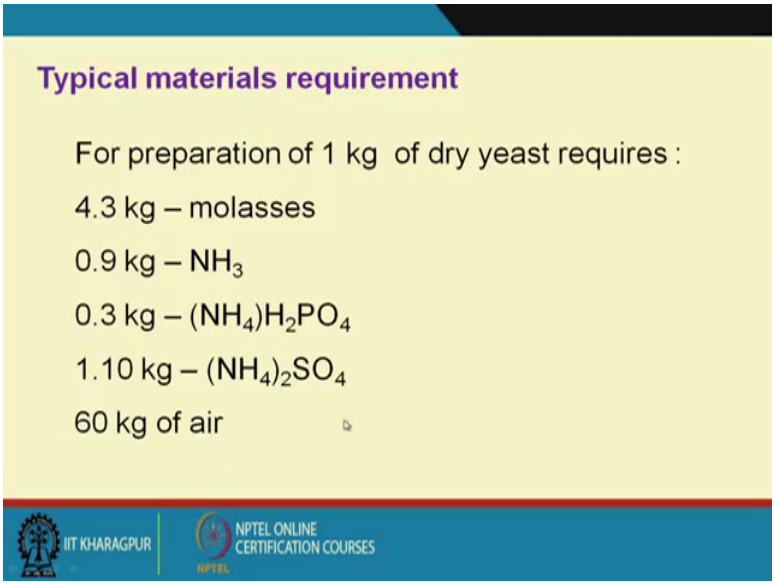

Now typical material analyses has been done and it has been found for the production of 1 kg of dry yeast required 4.3 kg molasses, 0.9 kg ammonia, 0.3 kg ammonium dihydrogen phosphate, 1.1 kg diammonium sulphite and 60 kg of air.

So this is the requirement basic requirement for 1 kg of dry yeast production now you can remember that initially I should you some stoichiometry of the bioprocess, now I want to try to show you how from the stoichiometry we can analyse this kind of that whatever that requirement we have molasses for this Bakers yeast production whether we can correlate with the stoichiometry.

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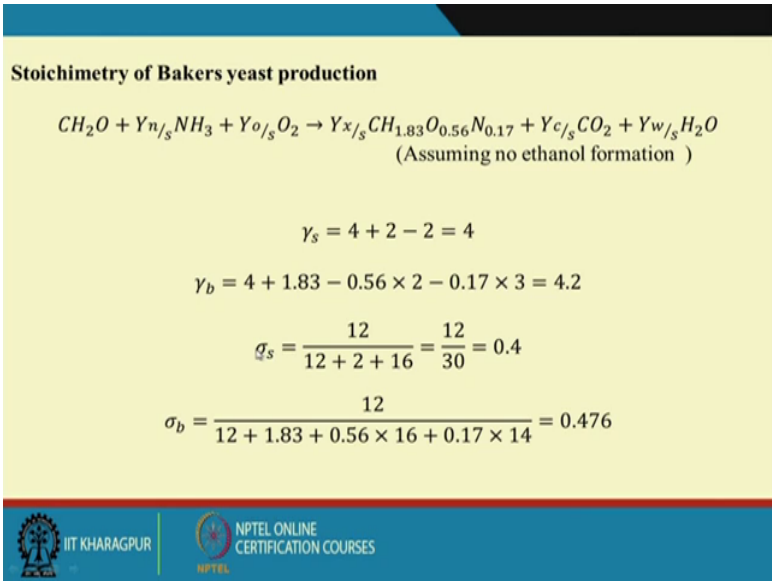

Now now you can remember that stoichiometry is like this this is this is the one gram atom of carbohydrate carbohydrate we understand that carbohydrate the name signifies that carbon and water that is the that that that should be present there so it is C6 H12 O6 that if you consider glucose and if you divide by 6 then you will get this, 1 gram atom of substrate and then you required let us assume Yn by s that is ammonia and Yo by s oxygen this gives the Yx by s cell mass this is the cell mass the molecular let us assume this formulae of Bakers yeast like this and Yc by s is carbon-di-oxide, Yw by s is water. Now if you do the degree of reductions of substrate and degree of reduction of of biomass is coming 4 and 4.2 and sigma s, sigma b is like this.

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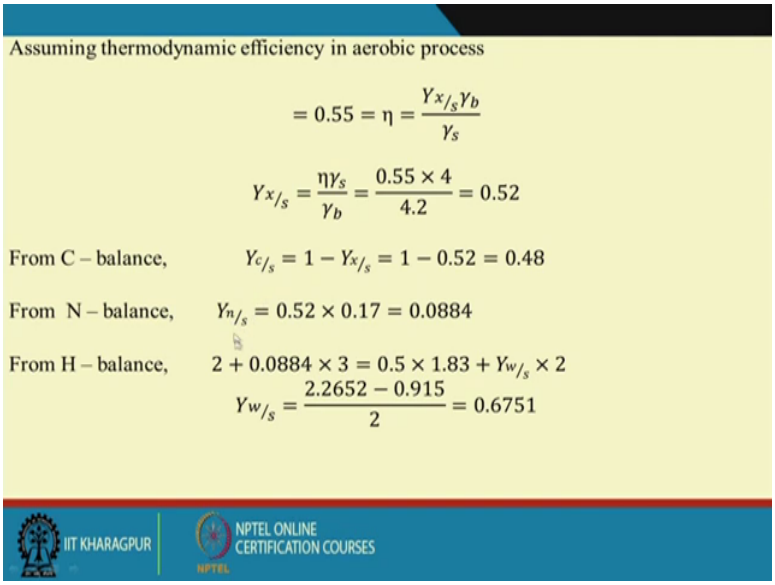

And we know now we thermodynamic efficiency of the aerobic process we know it varies from 0.5 to 0.6 now if we assume this value in between that 0.55 then eta this will be because epsilon p would be 0 because we assume here because we assume low ethanol production because this is aerobic fermentation process under aerobic condition no ethanol production takes place.

So we can we can assume the eta equal to this is Yx by s that gamma b by gamma s, so Y x by s we can easily calculate to 0.52 now if you do the if you once you get this value then we can we can find out that that other value we can easily calculate that we can we can find out Yc by s, Yn by s, Yw by s we can we can calculate.

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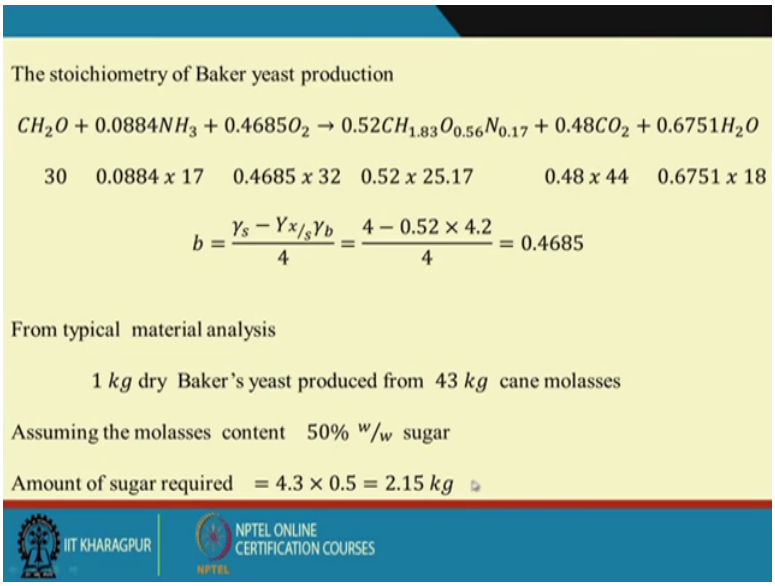

And we can put it in this equation like this that and and when when b value we can also calculate then then we know that 1 kg just now we we find out before that for 1 kg dry yeast we require 4.3 kg molasses So what we have done that 1 kg 43 kg cane molasses let us assume it contains 50 percent weight by weight sugar so sugar content is 2.15 kg.

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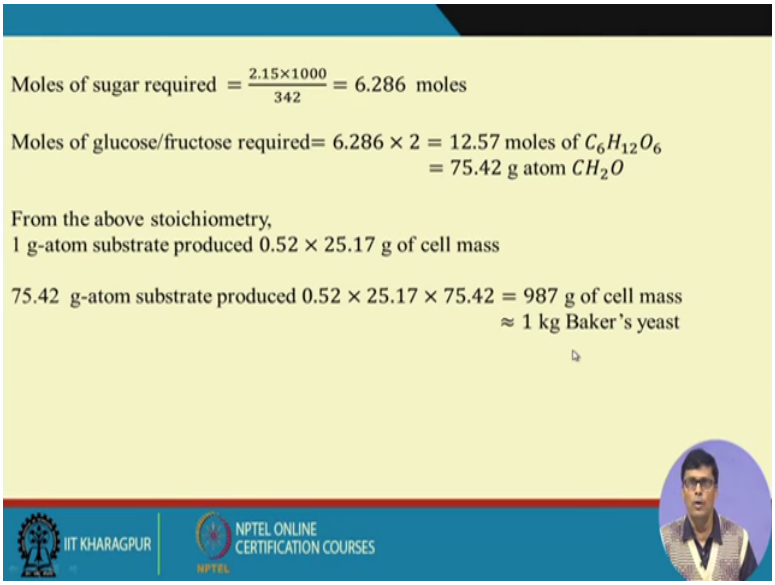

Now 2.15 kg sugar multiplied by 1000 then it will be gram this will, previously it was kg so previously it was kg and 1000 gram divided by 342 the molecular weight of sugar is 342, 6.28 moles of sugar is there. Now 6.28 moles sugar can be produced 2 moles of glucose and then you multiply this much of glucose, now this much of glucose 1 mole of glucose contains 6 moles of CH2 CH2O you multiply by this 6 we will get this the atom.

Now 1 gram atom substrate produces now you see that our stoichiometric equation 1 gram atom substrate produces 0.52 grams of this if it is like this then then what we can write 1 gram atom substrate produce this come of cell mass. This gram atom of substrate produce how much this is coming about 987 gram per gram of cell this is approximate to 1 kg of Bakers yeast. So what I point want to point out that stoichiometrically we can also justify that how how this amount of cane molasses is used for the Bakers yeast fermentation process.

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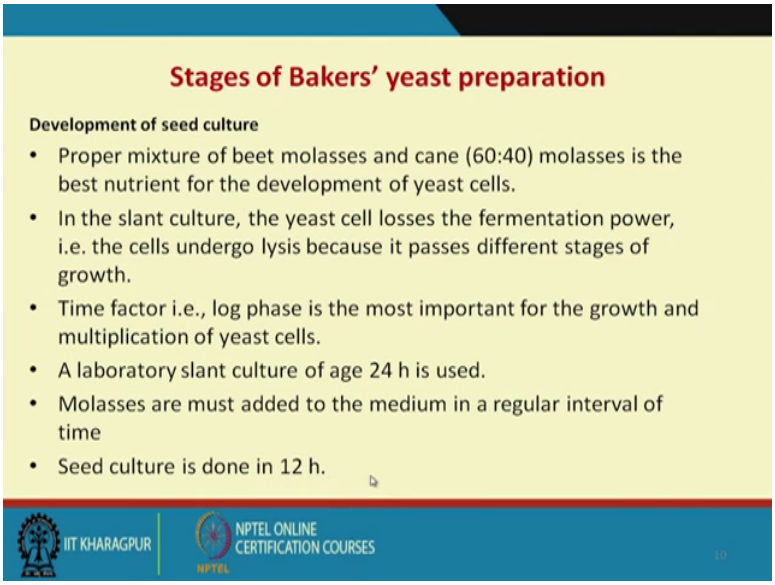

Now let us let us talk about the the stages that we have in the Bakr's yeast fermentation process, one is the development of the seed culture, proper mixing of beet molasses and cane molasses I told you 60: 50 50:50 they they some cases they find 60:40 the base nutrient for the development of the yeast cell in the slant culture the yeast cell uses the fermenting power that is the cell undergo lysis because it passes through the different stage of growth. The time factor the that is the log phase is the most important growth and multiplication of the yeast cells.

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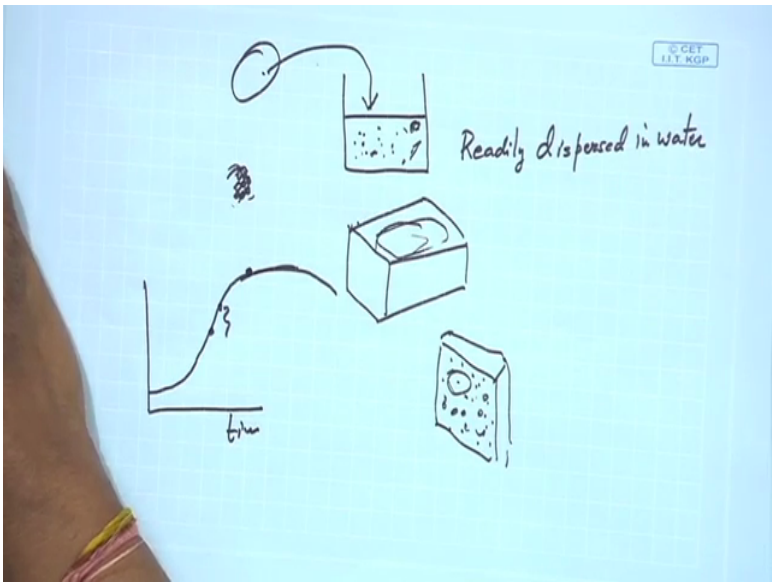

I I told before several times that whenever we do the inoculation that we we always considered the go cycle of the cells and this is the mid log phase and this is the lad log phase

and we always do the inoculation in between this phases so in between that if we if we if we do the inoculation here naturally number of dead cell will be more as so you know your rate of growth of the organism will be affected to a great extent.

The time of growth is very important log phase the most important for the growth and multiplication of the yeast cells, laboratory slant of 24 hours is used. Molasses are must be added to the medium and the regular interval of time and seed culture is done in 12 hours.

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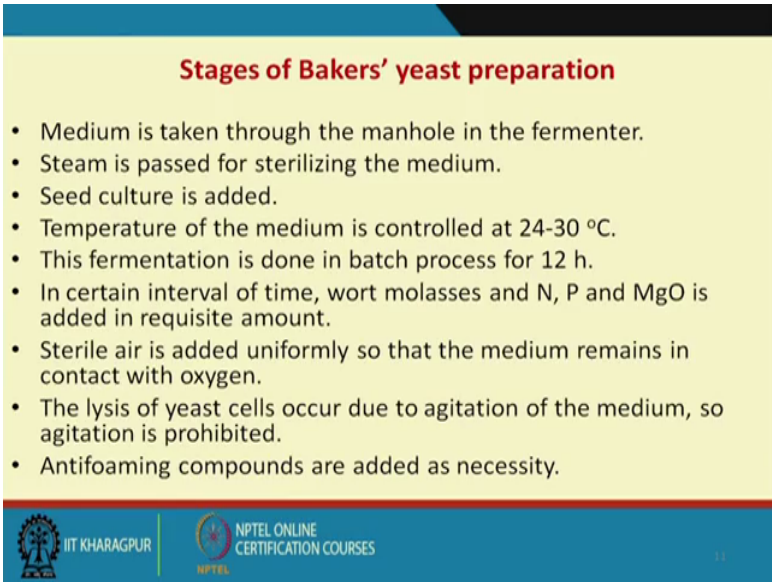

So this is the requirement we have then medium is taken through the manhole of the fermenter I told you that in the fermenter in the.

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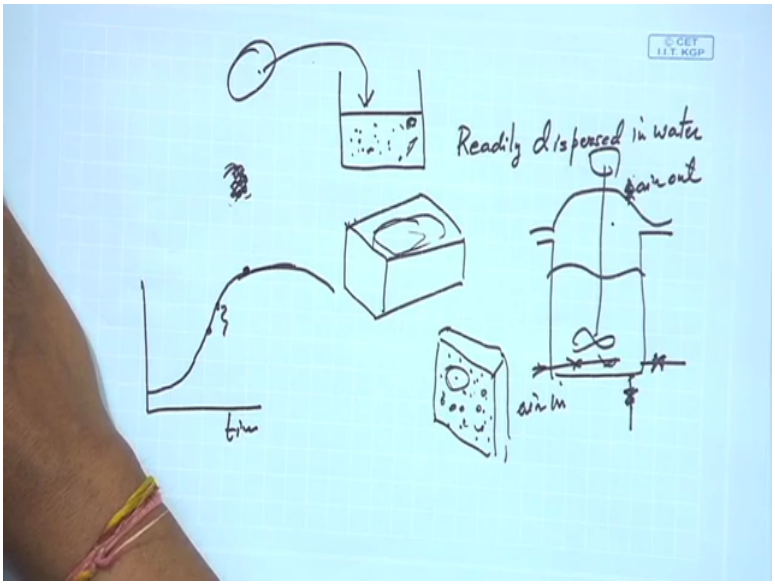

Suppose this is this is this is the fermenter and this is the aerobic fermentation process when when we carry out this fermentation that this is the aerobic fermentation process so what you this is air out this is air in we we close this value, if you close this value then what will happen the air pressure will build up take place then there will be manhole that that is that there are at the bottom. You take the material out and that is medium is taken through the manhole and here manhole we have gate value I have already shown you what is gate value, what is gate value and other type of value. The gate value is used in case of manhole then steam is passed through the for sterilizing the medium.

The seed culture is added then temperature of the medium control 24 to 30 degree centigrade this fermentation is done in the batch for 12 hours in certain interval of time oat molasses and nitrogen phosphorous and magnesium oxide is added in decosit form. Sterile air is added uniformly so that the medium remains in contact with oxygen, the lysis of the cell occurs due to the agitation of the media. So agitation is try to not prohibited we we try to control the agitation speed so that Lysis because one positive side with the positive thing with a yeast cell, yeast cell has very thick cell wall so even you increase the agitated speed the growth will not be muchly affected but we cannot go beyond after certain level usually 200, 250 rpm is good enough for the keeping the cell in suspension antifoam compound is necessary antifoaming compound is necessary whenever is required.

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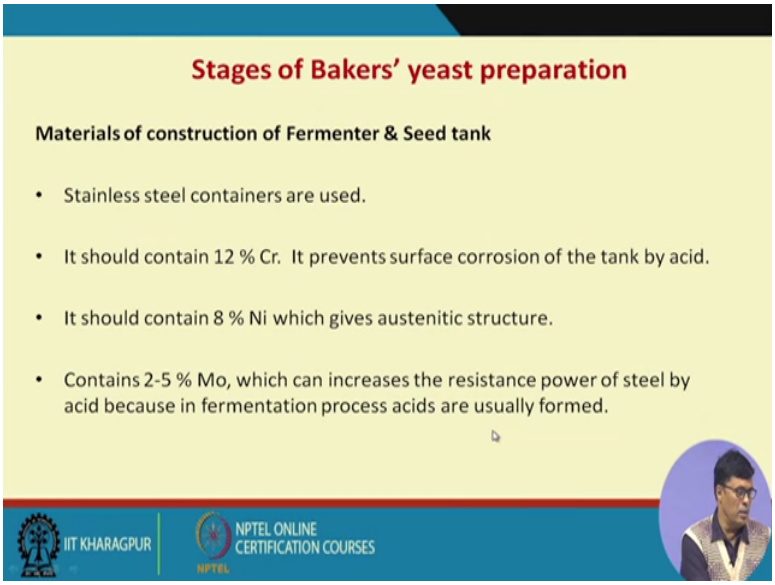

Now material of construction of fermenter I already explained. The stainless steel containers are used, different types of stainless steel that is available in the market I told you the SS304 that is largely used for our for our in the in the home made utensil and in the chemical industry we usually use SS3104 also we use in the biochemical industry usually we use the SS316 because most of the fermentation process, it produces lot of organic acid which is corrosive in nature.

So naturally that helps us to to develop that so there you know the resistance property of the construction material should develop, now here I have given you some instances that it should contents 12 percent of chromium it prevents the surface corrosion of the tank by acid, it should contain 8 percent of Nickle to give the austenitic structure austenitic structure means smoothness of the of the metal structure. If the if the if the surface of the metal structure is little bit rough then what will happen the cells will grow on the surface of the material of construction that is undesirable.

We want that all cell in case of starrer tank reactor all cells should grow on the on the liquid itself not on the on the surface of the container so this is requirement 8 percent Nickle is required to give the austenitic structure then 2 to 5 percent molybdenum which can increase the resistance power of steel by acid because in fermentation process acids are usually formed, so this is the how we can increase we can improve the quality of the stainless steel by changing the different metal content in the alloy steel.

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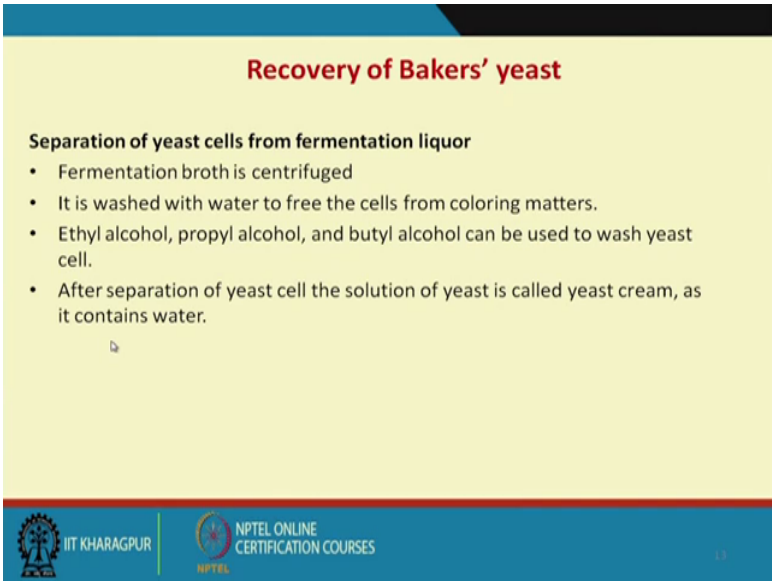

Recovery of the Bakers yeast separation of the yeast cell from the fermentation liquor, the fermentation broth is centrifuge or it is washed with water to free the cells or colouring matters the ethyl alcohol, propyl alcohol, butyl alcohol can be used to wash the yeast cells that after separation of the yeast cells the solution of the yeast is is called the yeast screen or it contains water.

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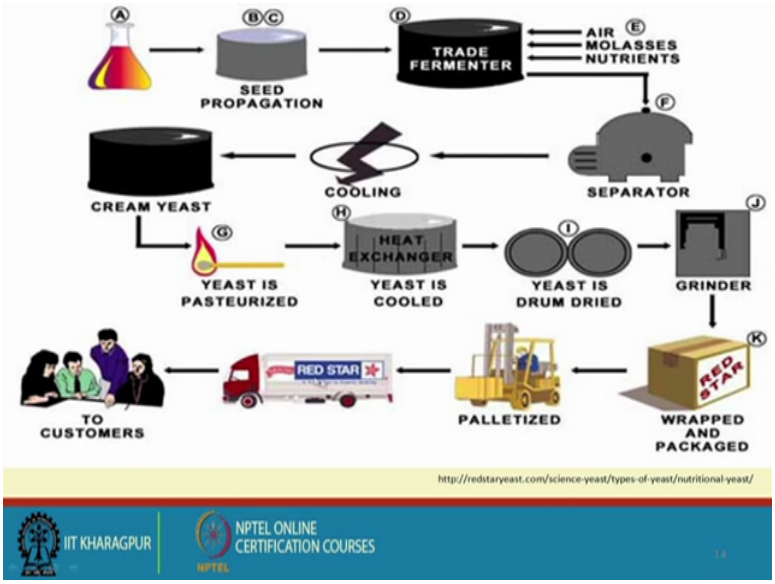

This is the process flow diagram I can explain this is the the conical flask where the cells are cells, cells grows here then cells propagate in the in the inoculum vessel then then it comes to the production fermenter in the production fermenter we give the we take the molasses we take nutrients

I told you you have to take nitrogen source we have to take phosphorous source we have manganese oxide then air you have to take because this the aerobic process after that you do the separation of the yeast cell then you have to cool it down cream of yeast cells then you pasteurize the yeast cells then heat exchanger again you cool it then you drum dry grind it and palletisation and sell it in the market.

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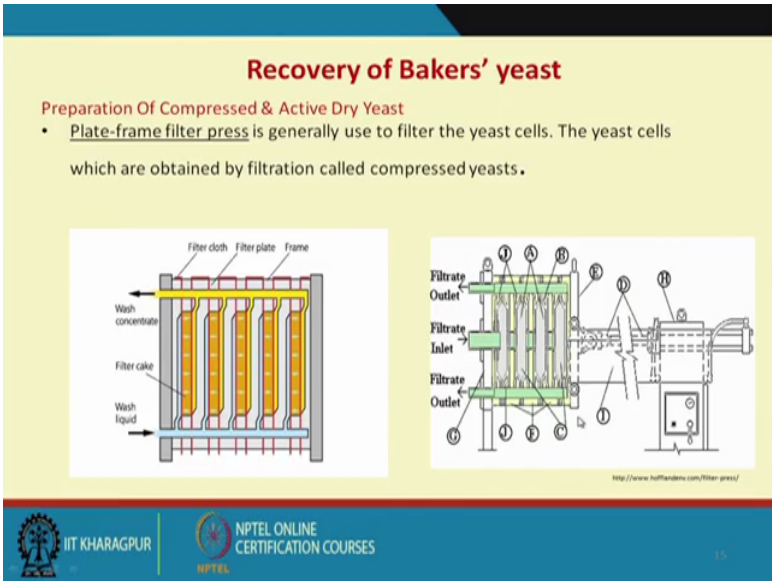

This is usually produced this is by the active dry yeast but I mentioned that here the centrifugation that is used for this to separate the yeast cells but that is not in practice in Bakers yeast industry because in the lab we do it in the centrifugation but in the Bakers yeast industry we use the plate and frame filter press because here here I can explain that you know that this is the cloth, you can see this is the this is cloth that we have fine cloth that app on the surface and and then this is the frame that we have and we pass the liquid like like worst liquid you pass this when we pass this then that liquid passed through this that here we collect the liquid and the solid material will trap here.

The Bakers yeast will will will trap here and then and this all the all the this is the that you know it is plate and frame filter press I I showed also that frames they punch together and they are wrapped with the thick wrapped with the thick cotton pad thick cotton pad you know thick cotton pad.

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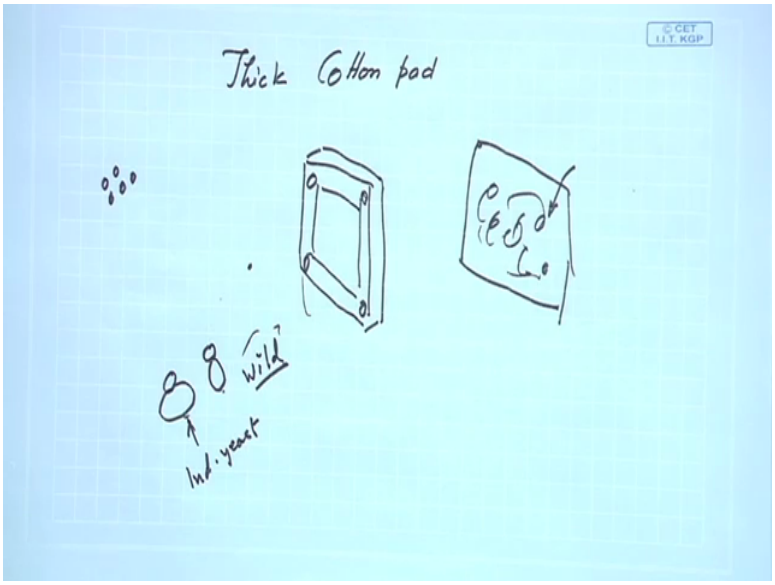

So they they because this frames frames looks like that I showed you before also it is just like wooden frame we have in the houses the wooden frame is looked like this so this wooden we are bunched together we have several and in between that we wrap the surface with this with this and we can we can make a hole and with the help of nut and bolt that is screw we can tight this and this is how it is run then yeast cells will accumulated here and then we when it is totally filled up we open this and collect the yeast cells.

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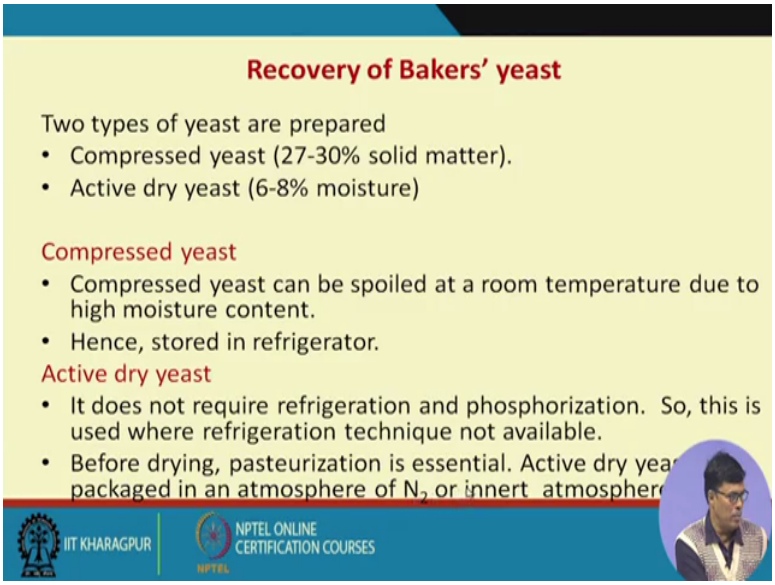

Then two type of yeast are prepared I told you compressed yeast and the active dry yeast compressed yeast contains about 27 to 30 percent solid matter and active dry yeast about 4 to 8 percent I told it is 6 to 7 percent. It is usually 4 to 8 percent and compressed yeast can be

spoiled at room temperature due to high moisture content hence it is stored in refrigeration and active dry yeast it does not require refrigeration or phosphorization so this is used when the refrigeration technique not available and before drying pasteurization is essential active dry yeast are packed in an atmosphere of nitrogen and inert atmosphere.

Here let me tell you that that dry yeast the dry yeast powder that we pack it under under anaerobic conditions suppose you have polythene pouch here and when you put the put the dry and and then we pass the nitrogen here in nitrogen atmosphere we do this now if we if we if we allow some air to enter into the system then what will happen that it contains you cell contains Bakers yeast is contains some kind of fats and this fats in presence of air and moisture undergo density and gives the foul smell that is undesirable.

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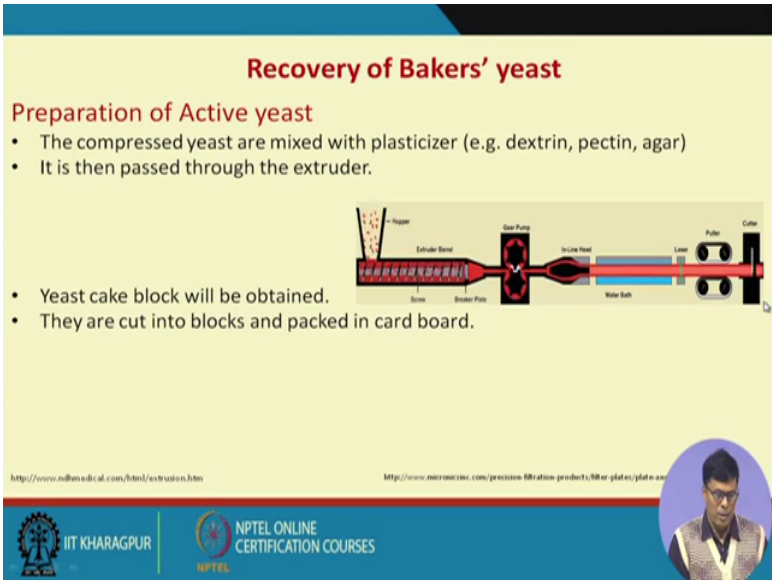

Now let me show you that how the active dry yeast formation take place it is very interesting that compressed air is mixed with a plasticizer I I told it this is the binder, binder is required otherwise the particle cannot hold together this the whole problem we use the dextrin, pectin and agar this is largely used then it passed through the extruder, extruder you can see that this is this is the screw conveyer, this the yeast is coming and screw conveyer is coming and it passes through the small orifice (())(27:12) this is called extruder that is going like this. This goes in the form of thread actually and then then yeast cake block will be obtained you can you can you can you can form a block or then you can you can cut it because this thread you can cut it here so that you can have small small that you know portion then you dry it.

When dry it then it forms a pebble the small small pebble formation should be there that is why we have seen at the initially the active dry yeast that that is not in the exact power form but in the pebble forms and then it is same to the cold storage of room carefully dried.

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And the contamination the contaminant during the Bakers yeast production acidic pH is maintained, media contains lactic acid contains lactic acid is used. Lactic acid is used as a preservative, acetic acid bacteria and some petrifying bacteria produce are major contaminants in this particular process here I I want to mention the petrifying bacteria in the day to day life we know that when you go out and you know some dead animal is scoring in some summer place we get very foul smell and this foul smell is mainly coming from due to the presence of the petrifying bacteria that they reproduces lot of protease enzyme they degrade the protein molecule and when they degrade the protein molecule they produce different types of organic acid and different types of acid their responsible for the formation of this foul smell.

So this is exactly what is mentioned that you know that putrifying bacteria produced are the major contaminants and detected by order, so you can you can have the foul smell after that wild saccharomyces cereviciae strain has less fermenting power hence contamination should be avoided.

This you can I showed you before also that this you can find out in the microscope very easily suppose your industrial microorganism is looking morphology is like this, so if when you when you observe from the microscope if you find that other other yeast cells are like

this so you can you can usually you can only differentiate that this yeast is different from this yeast so you can easily find out the east contamination if this this is considered as a this this we consider as a industrial yeast industrial yeast and and this is called wild yeast.

So wild yeast is very slow growing so naturally that will affects your affects the quality of yeast to a great extent and fermentation process to a great extent. All pipes as well as the tanks should be properly sterilized. Air pipe contains cotton wool and air is passed through the pipe to sterilize, sterilize it.

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Now quality control test also very important as per the the Bakers yeast fermentation is concerned the solid content because because here I want to point out very important thing that any biochemical industry that quality control test three different sections is very important.

One is raw materials another process another is a product. The raw material if the raw material is not properly maintain then your product will not be proper, so raw materials you have you have a chart that you find out exact what raw material you want so when you use the raw material for the production fermenter you have to check thoroughly whether is coming within this range or not and then you have to go through the process in the process we have to find out whether you maintaining proper temperature, proper pH, proper operating condition, reserve oxygen concentration and other the antifoam oil.

So that you get the proper product and also quality consult is in the product you have to have different composition whether it is whenever you because as per the Food Product

Organisation it is mandatory that you have to give the composition of the food whenever you do any do any marketing. So naturally that until and unless your your your raw materials is quality is maintained and process control is not maintained you cannot expect the better quality of product.

So this is done on the basis of solid content, colour, appearance, dispersion in water already I mention, nitrogen and phosphorous content. Fermenting power means that how how quickly it produce it ferments the the substrate that is very important now more more if the high fermenting power your your time required for the fermentation process will be less otherwise it will take longer period of time. Package weight and backing test, backing test means how much carbon-di-oxide is produced that you know that also that is very important, if it produce carbon-di-oxide very slowly then incubation time for the for the bread making industry after the dough preparation that will be very very high that is undesirable.

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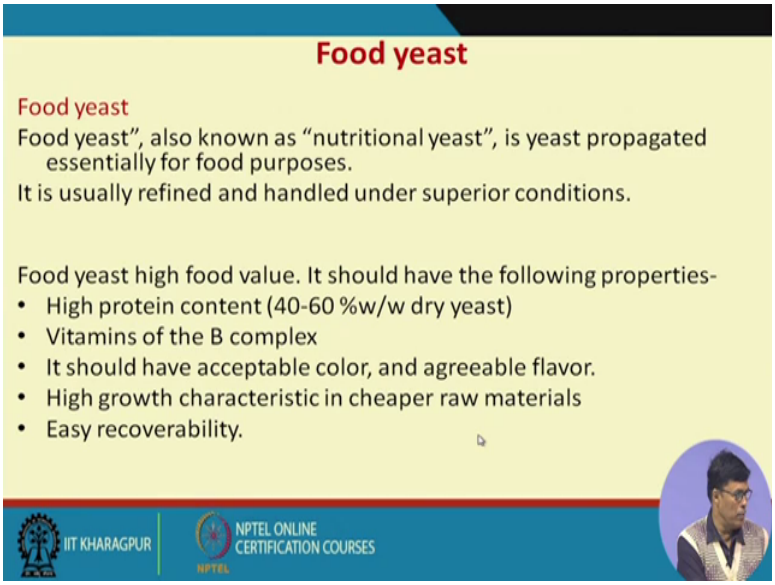

So so food yeast this is this is also consider the food yeast this is the food yeast means this is called nutritional yeast it is a propagated essentially for food purpose because here I want to mention that I think couple of years before the yeast is considered as a good source of vitamin T, vitamin B because doctor they recommended this yeast tablet when we have some deficiency in our in our body that the vitamin B is recommended, now it is usually for refined and handle under superior condition food yeast to the high food value. It should have the following property, high protein content, vitamins for B complex and it should acceptable colour and agreeable flavour, high growth characteristics and cheaper raw materials, easy recoverable.

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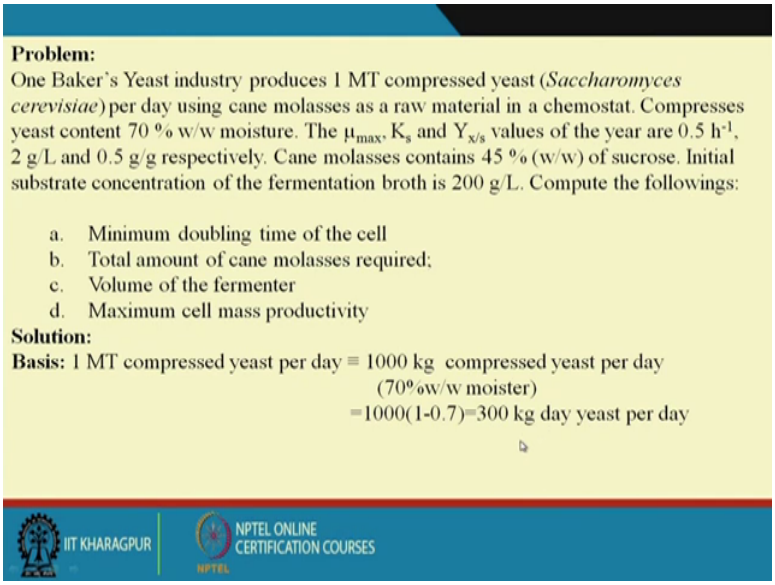

And and here I want to show you one small problem I hope this is very interesting as per as per Bakers Bakers yeast industry is concerned. The problem is very simple one Bakers yeast industry produce 1 metric tonne of compressed yeast saccharomyces cereviciae per day using cane molasses as a raw material in a chemostat, chemostat means continuous starrer tank reactor.

The compressed yeast content 70 percent weight by weight moisture, Mu max K and Yx by s values of the of the yeast this is the wrong yeast are 0.5 hour inverse 2 gram per litre and 0.5 per litre respectively. Cane molasses contains 45 percent weight by weight sucrose, initial substrate concentration of the fermentation broth 200 grams per litre. Compute the following.

Minimum doubling time of the cell. Total time total amount of cane molasses required which is largely required in the fermentation industry and volume of the fermenter and maximum cell mass productivity. 1 metric tonne of compressed yeast means 100 kg of compressed yeast per day and since it contains 70 percent moisture we can assume it contains 30 percent total solid, so if you multiply by 0.3 you will get 300 kg of dry yeast per day.

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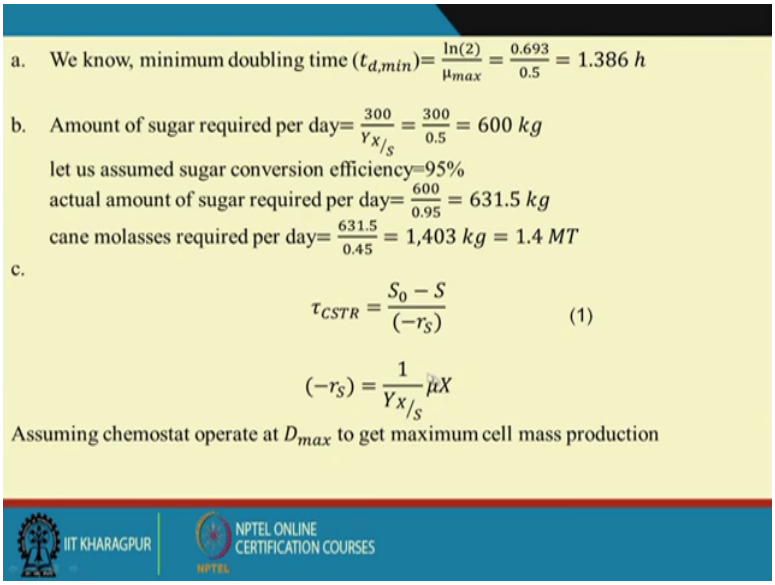

The we know that minimum doubling time is that ln2 by Mu max, so you can easily calculate this and the amount of sugar required you can easily also calculate if you know this the 300 kg of that cell mass we have to yeast we have to produce Yx by s you know that is a gram of each cell produced per gram of sugar then this is 0.5

So you require 600 kg of sugar, if I assume 95 percent conversion efficiency actual amount of sugar required is 631.5 kg and if cell molasses contain 45 percent of sugar if you divide by 0.45 you will get 1.4 metric tonne of cane molasses required. Now if you want to calculate the volume of the fermenter required we know (())(36:38) CSTR equal to S0 minus S by minus rs, minus rs equal to I can I can write like this.

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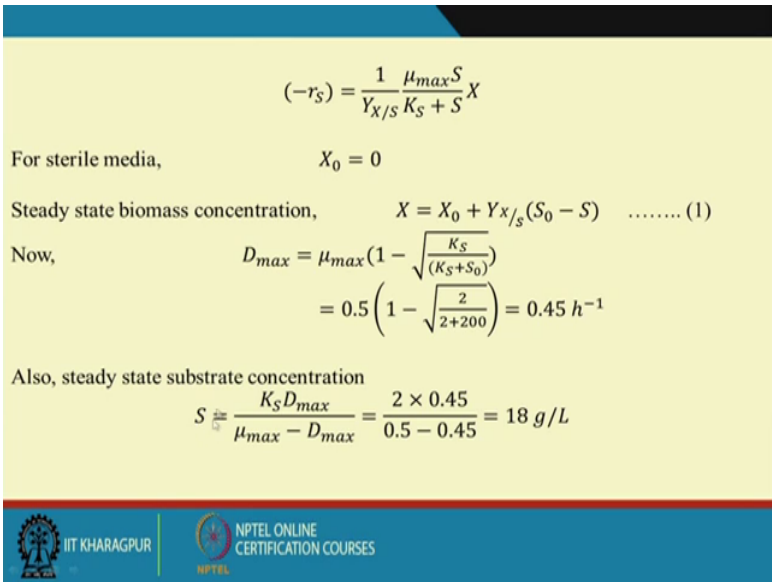

And we calculate the Mu max value from the Mu max value we can find out the X value, D max value we can find out from D max we can find out the S value, S value means under what is D max D max has the dilution rate when will get the maximum cell mass formation then maximum rate of cell mass growth and then that that condition what is the substrate concentration we find out.

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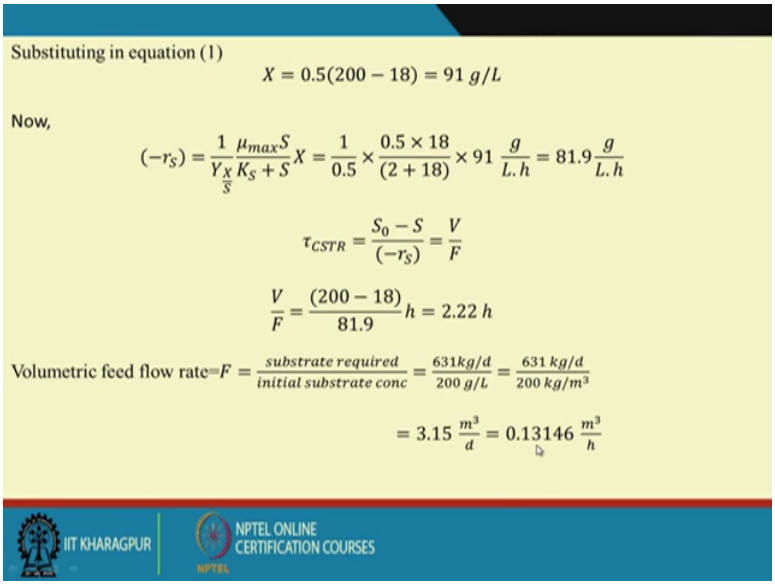

And this is the cell mass concentration and the from that we calculate we we find out minus rs value, what is rate of substrate consumption this is coming about 81.9 gram per litre per hour and from that you find out (())(37:31) CSTS space time for this is 2.22 hours. Then volumetric feed flow rate is substrate required by initial substrate concentration this is coming about 0.13134 cubic metre per hour.

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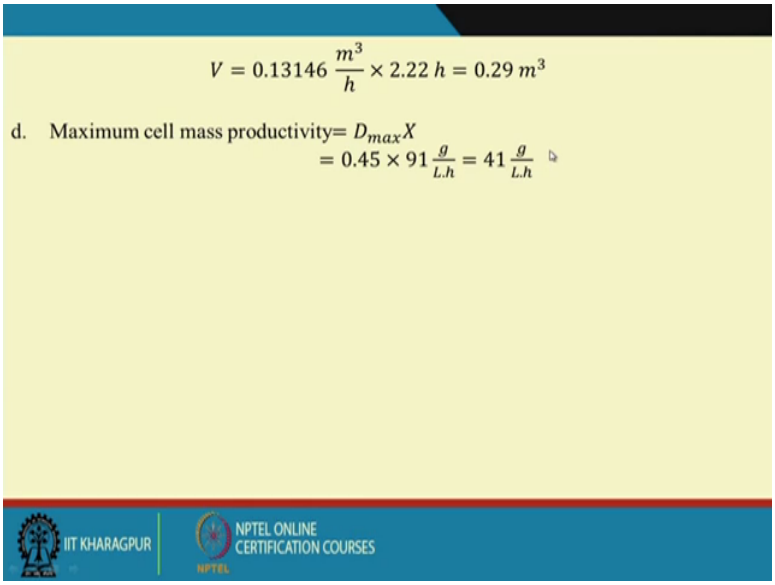

So volume of the fermenter is coming about 0.2329 cubic metre maximum productivity we can easily calculate we know D max, we X value if you multiply with this we can find out what is the maximum rate of cell mass production. So this is this is this is all about about the Bakers yeast fermentation process we try to discuss the process in details and try to also give some mathematical analysis of this particular fermentation process, thank you.