Course on Industrial Biotechnology
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Lecture 28
Module 6
Ethanol Fermentation (Continued)

Welcome back to Industrial Biotechnology course. Now I was discussing about the ethanol fermentation process though in the last class I tried I discussed that that how the ethanol production takes place what are the different types of raw material is used for ethanol fermentation process and that we have two type of alcohol, tax alcohol, non-tax alcohol then we discuss distilled liquor and non-distilled liquor.

Now in this presentation I am going to go little bit in details and at the end we also discuss some advance ethanol fermentation process at the end I shall discuss one problem related to the ethanol fermentation process.

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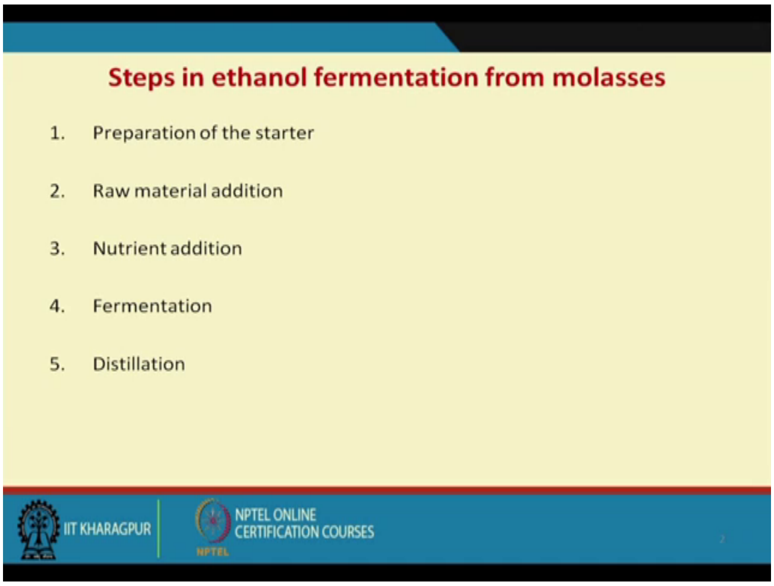

Vitamins content in molasses

	Biotin (mg L^{-1})	Inositol (mg L^{-1})	Pantothinol mg L^{-1})
Beet molasses	0.09 – 0.13	800 - 5700	50 – 110
Cane molasses	2.7 – 3.2	600	50 – 60

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Now the question comes what are the steps involved in the ethanol fermentation process from molasses I told you that molasses is considered as a very effective raw materials for the ethanol fermentation process.

Now why the molasses contents is considered as a very good raw materials for the ethanol fermentation process, the reason is that it contains the vitamin like biotin, inositol and pantothinol and if you look at the vitamin distribution in case of beet and cane molasses. In my previous one of my previous lecture I mentioned that cane molasses is largely available in India and beet molasses is mostly available in the western country. Though in India also in south India in south part of India some cases some places we produce beets and from that also we can produce beet molasses but number is very few.

And if you look at the vitamin distribution in beet molasses and cane molasses the biotin, inositol and pantothinol that plays very important role in the alcohol fermentation process.

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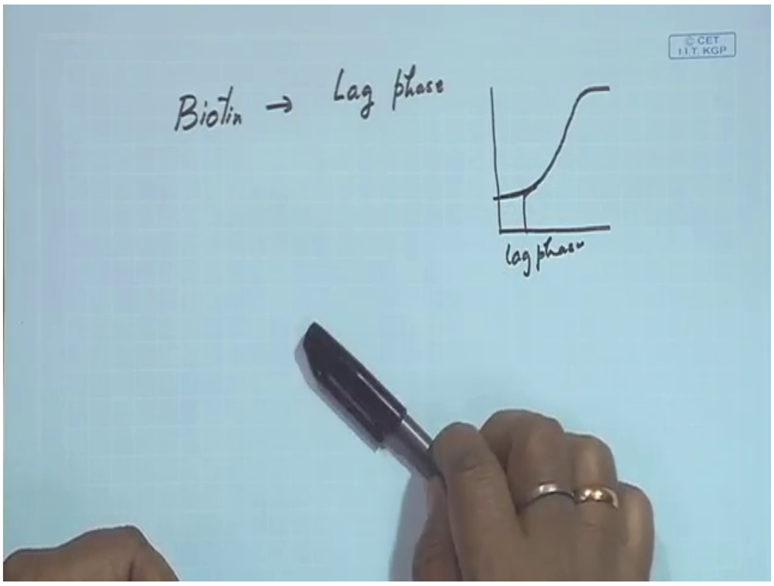

Now if you look at biotin as for example, if your media does not contain any biotin then lag phase of the fermentation will increase lag phase of the fermentation increases that is undesirable. You can remember when I discuss the discuss the growth cycle of the cell I told that this is this is considered the lag phase.

This lag phase should be as small as possible if you have we have very less lag phase then we our fermentation time of fermentation can be reduced drastically, so biotin is a very in presence of biotin this lag phase can be reduced. So this is the essential component present required in the fermentation broth then inositol if the inositol present then your absent then your organism relax to ferment sugar that is undesirable so this is the so this is another and pantothinol if pantothinol does not present then your organism easily undergo the autolysis that is undesirable.

So if you if you look at this vitamin in case if beet molasses in case of cane molasses their percentage is varies that in case of cane molasses the biotin content high in case of beet molasses biotin content competitively less but other vitamins are quite significant. So it is recommended that if we mix beet molasses and cane molasses in the ratio 1 is to 1 then it is ideal for the alcohol fermentation process.

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So first in case of alcohol fermentation process first we shall have to prepare the starter. Starter is nothing but that is a culture you have to produce the culture and I told you that if you look at the volume of the culture usually 5 to 10 per cent of the fermentation media then raw material addition you have to add some raw materials the neutral addition if it is required then your you have to carry out the fermentation process then distillation.

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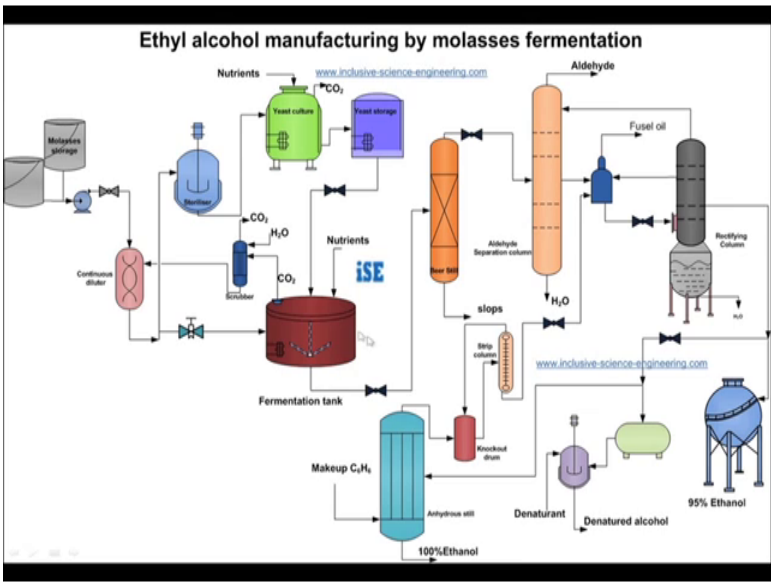

So this are the couple of steps involved in case of ethanol fermentation process. Now if you look at the process flow diagram of this ethanol fermentation process. This I have taken from this source and it is like this that the cane molasses I told you can remember that cane the cane molasses is a seasonal raw materials because cane produced in a particular season not throughout the year.

So you have the every industry they have molasses storage tank that just in front of their main gate and they huge very big tank and where this stood the cane molasses for whole years and then from that they with the help of pump and I told you that this sensor is a very viscous liquid. We use the gear pump the special type of pump used for dragging the viscous liquid from one from one place to others and we take it to the molasses measuring tank where before the fermentation we take the desired amount of molasses and then we dilute it then and add some nutrients then we put it to the fermentation vessel.

So it is like this, this is molasses storage tank and then then we take it here continuously we dilute this then we can we can do the sterilization because for the yeast inoculum preparation we shall have to sterilize if you when you go for this ethanol fermentation we do not require the sterilization directly this media can be used in the fermentation tank but the when you prepare the culture the culture is prepared under aerobic conditions so here we require the sterile media. And then yeast storage tank then from that we put it in the fermenter as inoculum. After the fermentation is over it passes through the beer still where heat this material passes though the distillation column and from the distillation column we can separate the ethanol, we get I told you 95 per cent ethanol but if you want to have 100 per cent ethanol then we shall have to use the benzene as a solvent though we cannot use water we can use benzene as a solvent then you can get the 100 per cent of the ethanol.

So this is this is like this we can add some kind of chemical to make the alcohol denatured this is how this is in practise in the industry.

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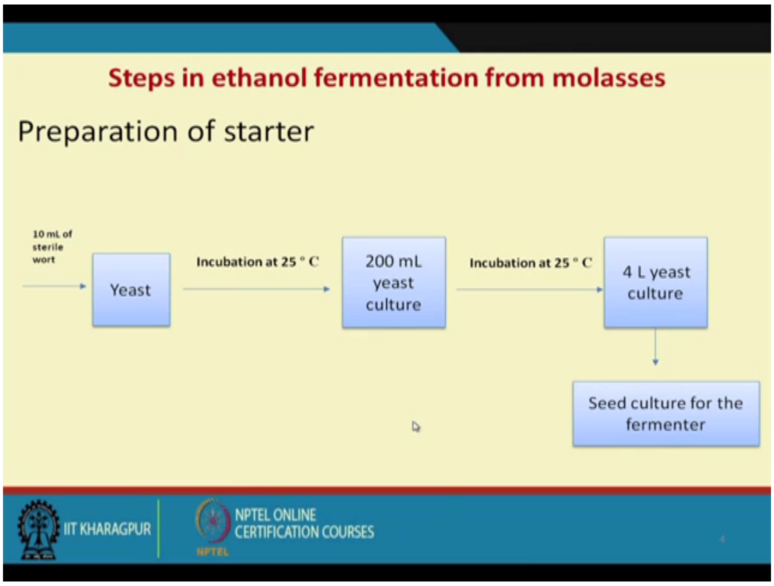

Now first step is the preparation of starter because we have 10 millilitre of sterile wort, wort is actually this is the it is liquefy starchy material and suitable for the fermentation purpose and this is where yeast is grow and then we incubate 25 degree centigrade then put it 200 millilitre of yeast culture then we incubate again at 25 degree centigrade to the 4 litre yeast culture then with this is culture we use in the inoculum vessel or produce the inoculum then finally we put it in the fermenter.

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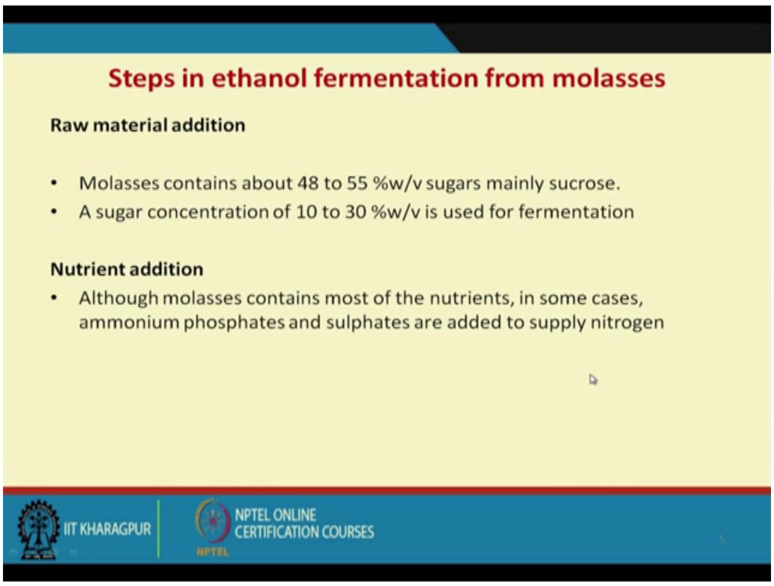

Now the raw material addition that molasses contains 45 to 55 per cent weight by volume that is sugar and sugar concentration of the fermentation broth usually varies from 10 to 30 per cent weigh by volume because initially that you know may be 15 20 years before that alcohol concentration of the fermentation broth was 7 to 8 per cent, now it is it is due to development some osmotolerant and yeast, yeast tolerant, alcohol tolerant yeast cells it is possible to increase the alcohol concentration up to 15 per cent.

So your your so the sugar concentration also increase to a great extent. Now nutrient although the molasses content most of the nutrient in some cases ammonium phosphate and sulphate are added to supply the nitrogen source.

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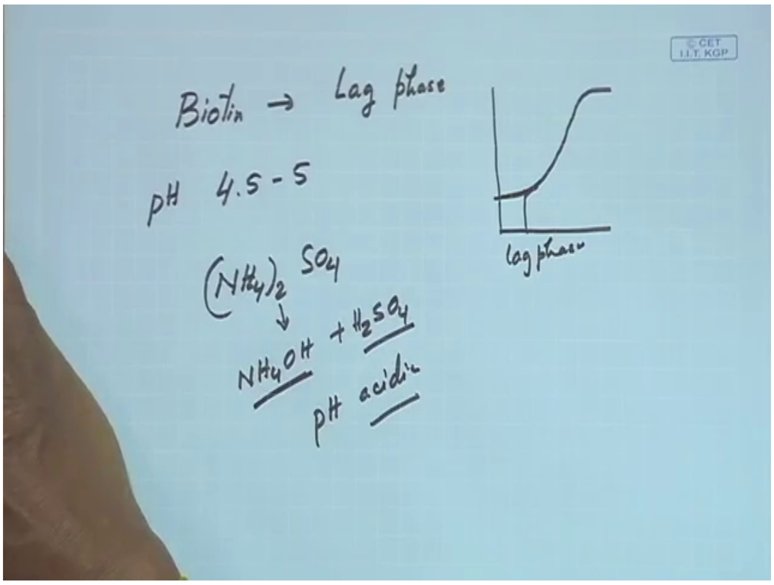

This is usually now here I want to point out one thing very interesting thing that I told you that in yeast fermentation process usually the usually maintain the acidic pH that is 4.5 to 5 this is the pH that we maintain.

Now you know ammonium ammonia diammonium sulphate is used when you use the diammonium sulphate make a solution we gave the ammonium hydroxide and H2SO4. So then since the H2SO4 is highly acidic as and this is the weak base, so when you make a solution of ammonium sulphate the pH will be acidic. So you do not have to add some acid to maintain the pH automatically the pH will be acidic

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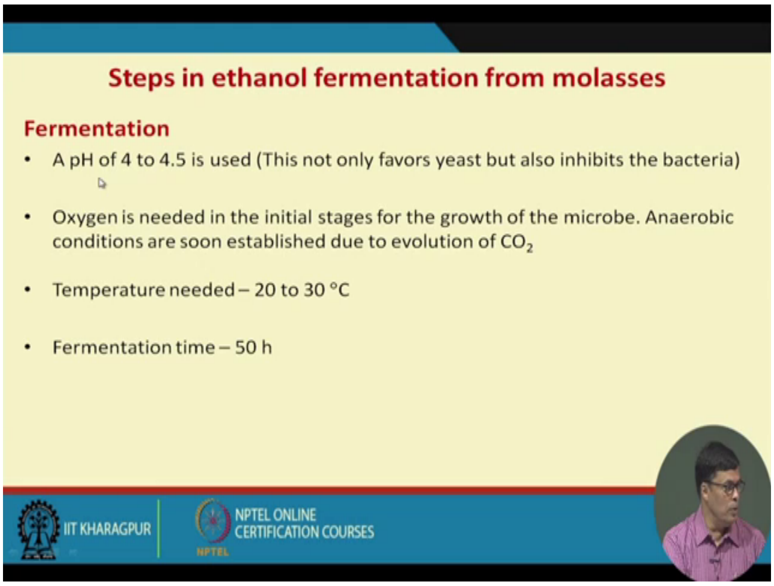

So that is why ammonium sulphate that usually prescribed as a nitrogen source greatly used for yeast fermentation process now that I told you the pH is 4 to 4.5 is used, oxygen is needed at the initial stage for the growth of the organism for the preparation of the inoculum then anaerobic condition then the anaerobic condition is established because in the fermentation process above the organisms used whatever dissolved oxygen present there for their growth then automatically the anaerobic condition that prevail that and due to the evolution of carbon-di-oxide, temperature 20 to 30 degree centigrade and fermentation time is 50 hours.

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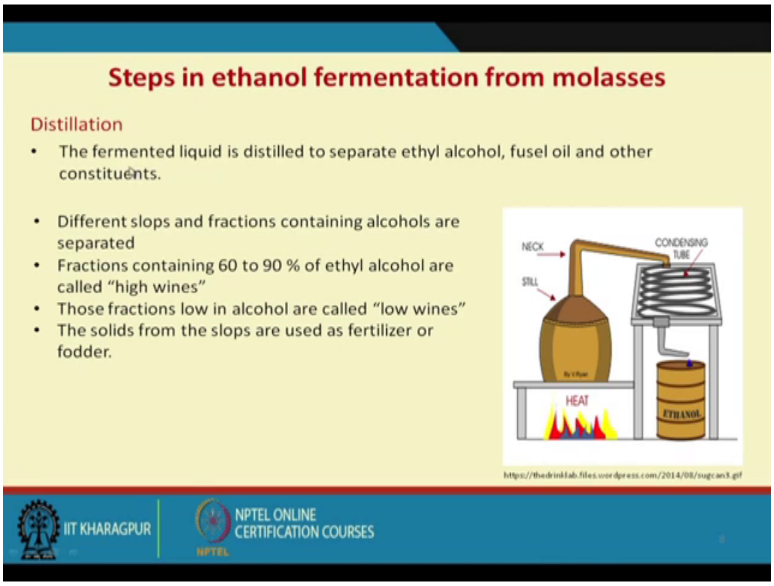

Now distillation that if you look at the look at the distillation that is the fermented liquor it distilled to separate the ethanol, fusel oil and other constituents present in the fermentation broth. I told you fusel oil nothing but higher alcohol like amyl alcohol, butyl alcohol. So different type of alcohol is produced this is the higher alcohol this causes the bitterness in the beer but this is very much useful when we use this as alcohol used as a power alcohol is increased the (())(11:42)

So different slopes and fractions contains the alcohol are separated fractions containing 60 to 90 per cent alcohol I call the high wine and those fraction low alcohol we call it low wine the solids from the slops are used as fertilizer and fodder because after the separation of the alcohol whatever things remain that is called stillage that can be used for either used as a fertilizer or fodder that can be used or this can be used as a raw materials for anaerobic fermentation process for the production of methane and carbon-di-oxide.

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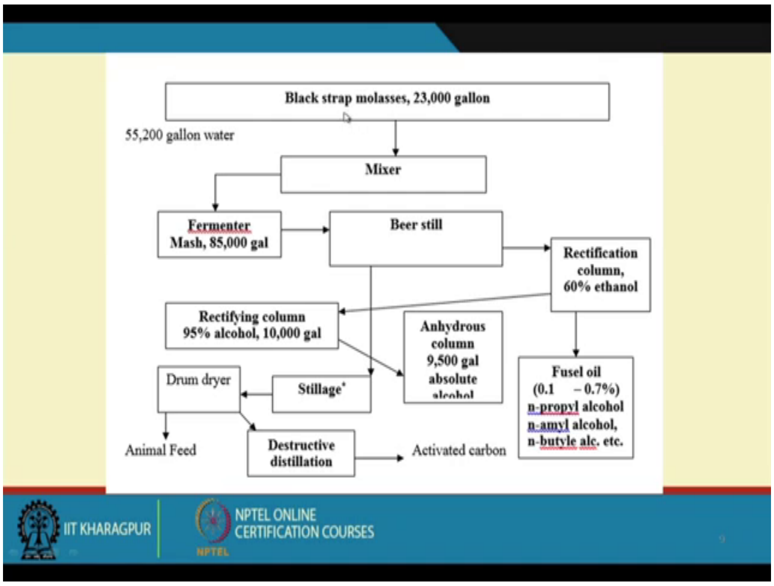

Now this is the this is kind of typical block flow diagram black strap molasses cane molasses we take it we mix it in the mixer with water with significant amount of water we add here and then we passes through the fermenter and from the fermenter the beer still and from the beer still we have rectifying column, rectification column we get 60 per cent alcohol further we passes through the rectification column we get the 95 per cent alcohol then we can produce the anhydrous column or we use the azeotropic distillation process by using benzene as a solvent we get the absolute alcohol.

Now in the beer still after the distillation of alcohol whatever the left we call it stillage you can drum dry and we can use as a animal feed or this is a when it undergo the distract distillation process it produces active activated carbon, now I was talking of the fusel oil. This alcohol during the distilled product we get some fusel oil the concentration of fusel oil is about 0.1 to 0.7 per cent. Normal propyl alcohol, amyl alcohol and butyl alcohol this has a different alcohol present in the this fusel oil.

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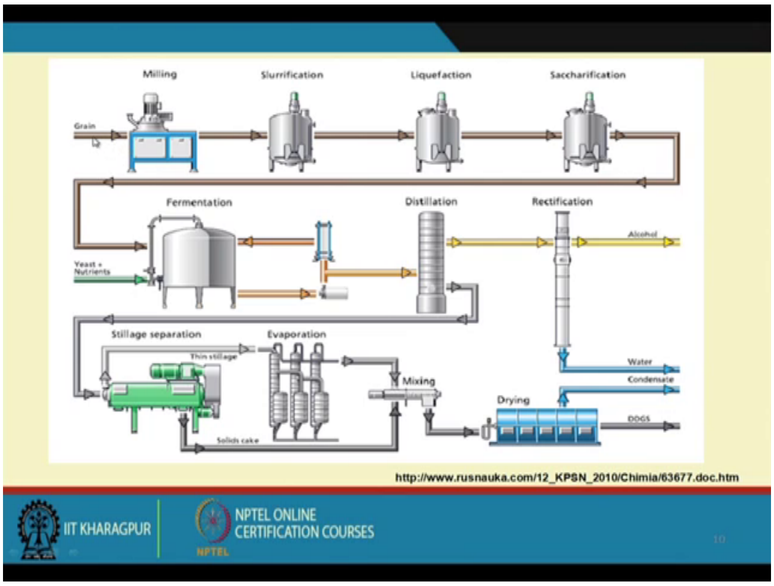

Now process flow diagram is like this we have grain because we have taken the example I told you that not only that alcohol produced from cane molasses but we use the broken rice the broken rice we can take it here we have the milling machine then we can slurry then liquefaction.

And not only liquefaction we heat it gelatinised the starch after gelatinisation we add enzymes here amylases enzymes for the saccharification and this saccharified material we have sugar we put it in the fermenter where we add some yeast and nutrient and when it undergo fermentation process and then we after the fermentation we distil this we pass through the rectification column we get the alcohol.

We take the water out and the stillage is coming this way, mixing and drying this is DGGS the distiller dry grain solid that also is used either it can be used as a very good fodder. It can be used fodder in the by different industry they use for that the fodder. So by adding some nutrient some other nutrient fortifying this with other components. So this is this is this is the typical process flow diagram of the alcohol fermentation process using the starchy raw materials.

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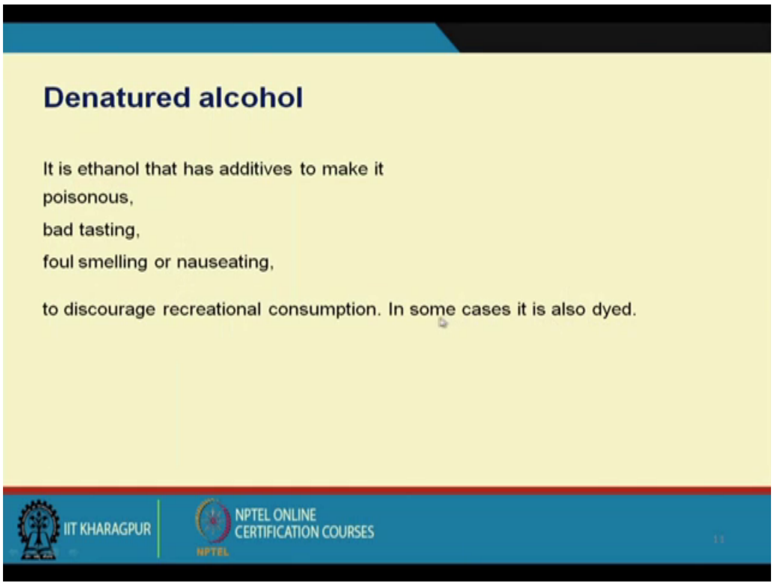

Now denatured alcohol I already explained that I want to discuss I do not like to discuss again but fusel oil I want to tell that this is 0.1 to 0.7 per cent of the cruse distilled spirit it is mixture of amyl and isoamyl alcohol primarily, smaller quantity of isobutyl and normal propyl and trace amount of acids, esters and alcohol. This is this is also used as a lacquer solvent I told you fusel oil also in in in acts as a octane number enhancer. So when you use alcohol as a as a power alcohol then it is desirable it will improve the quality of the fuel but when you use as a as a for human consumption then this is not desirable because this gives the bitter taste.

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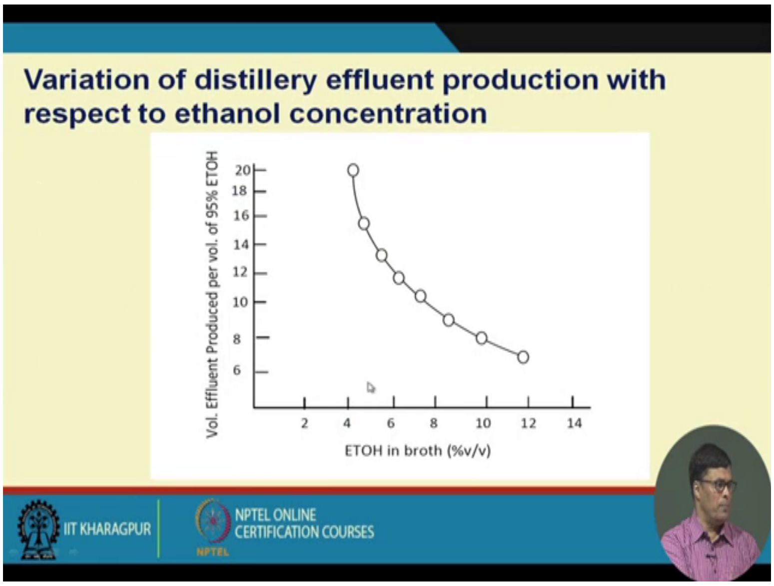

Now alcohol industry one problem is that this distillery that effluent that is produced here I want to discuss one very important aspect that I told you that the concentration of alcohol plays important role for the effluent production process. The correlation between the percentage of alcohol present in the fermentation broth and the volume of the effluent produced per unit volume of 95 per cent ethanol it is like this.

Now this clearly indicate that as the alcohol percentage increases the volume of ethanol production also drastically reduced. Now in early days because we have 15 to 20 years before in India most of the fermentation process has been carried out in the batch fermentation process and where we get 7 to 8 per cent alcohol, now here if you have 7 per cent so it is approximately equal to 12 to 13 litre alcohol per litre distillery effluent per litre of alcohol.

Now-a-days this is this has increases the ethanol concentration increases to 14 to 15 per cent and the volume is drastically reduced. So this is the this is the typical advantage of the of the efficient fermentation process. Efficient means with respect to higher concentration of alcohol production in the fermentation process.

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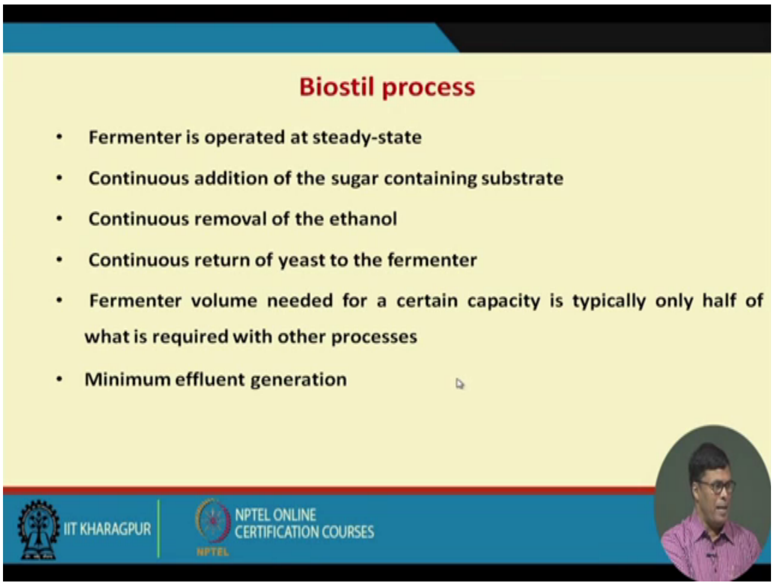

So Biostil is a kind of company they come with this continuous process to improve the ethanol production, now-a-days some industries in India they try to they are using this process because continuous process the alcohol productivity is much much higher as compared to batch process. Now in the biostil process the fermenter operate in a steady state I showed you this is the start time reactor in the start time reactor it is possible to maintain the steady state and steady state means the concentration under steady state condition the concentration remain unaltered, continuous addition of sugar, containing substrate and continuous removal of ethanol I shall explain the process with schematic diagram.

And then we have this continuous return of a yeast that take place. One drawback we discussed of the ethanol that CSTR that continuous cell mass bleeding from the reactor because we the continuous start time reactor we always we lose some cell mass. So to maintain the cell mass concentration constant we recycle back the some of the cell mass in the reactor to maintain the cell mass concentration constant the fermenter volume needed for a certain capacity is typically only half what is required for other processes because volume is drastically reduced and minimum effluent generation.

Now here I want to point out also the man power requirement in this particular process is drastically reduced about 10 per cent as compared to the batch process. So in that way this process is find very economical for the ethanol fermentation process.

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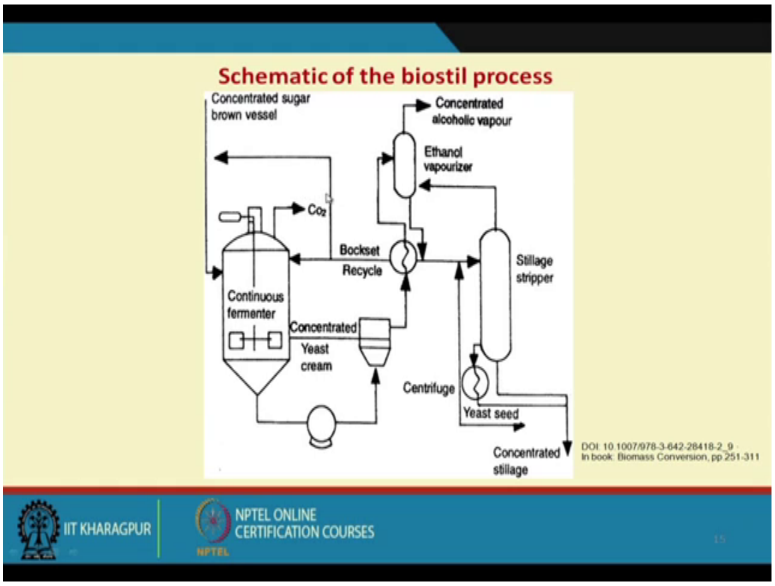

Now schematically this can be explained like this this is a continuous CSTR process we can see this is the starrer and this is your sugar your putting inside the system and continuously it produce carbon-di-oxide and then it passes through the centrifuge and this concentrated it goes to the centrifuge then concentrated cell you recycle back some cell you take it out and you take it out here.

And then we passes through the distillation process and after distillation process some of the liquid you use you take it back but the reason is that because I told you that cane molasses contains about more than 50 per cent or approximately 50 per cent of the sugar but sugar concentration in the fermentation broth is quite less. So what we have to do is dilute this cane molasses significantly the significant amount of water is required.

Now if you can recycle this this effluent then what will happen that effluent contains some unconverted sugar that will get an opportunity to ferment further and also water consumption in the fermentation broth will be reduced to a great extent. In this way we make the process they make the process very economical and amount of as I told you amount of amount of stillage production amount of effluent production is drastically reduced this is also one tenth as compared to the batch process, so the money that the expenditure involved for the waste water treatment process also significantly reduced.

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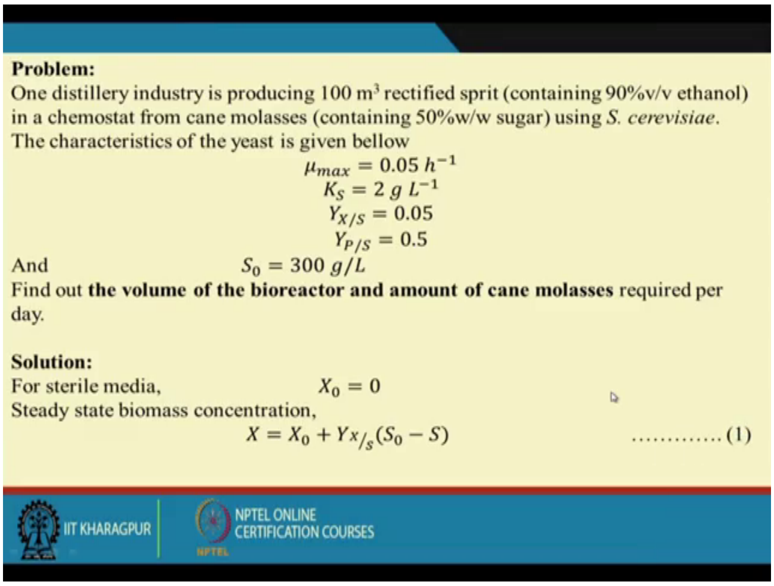

Now I want to discuss one problem with the distillery industry this is very interesting problem that suppose the question is one distillery industry it producing 100 cubic metre of rectified spirit containing 90 per cent of volume by volume of ethanol in a chemostat for from cane molasses containing 50 per cent weight by weight sugar using saccharomyces cerevisiae. The characteristics of the yeast cell is given here, okay this is under anaerobic condition, aerobic condition the characteristics of the organism will be different

So Mu max is 0.05 hour inverse, Ks is 2 gram per litre, Yx by s is 0.05, Yp by s is point p by s means ethanol gram of ethanol per gram of substrate consumed is 0.5 its 0 value is 300 gram per litre. Find out the volume of the bioreactor an amount of cane molasses required per day.

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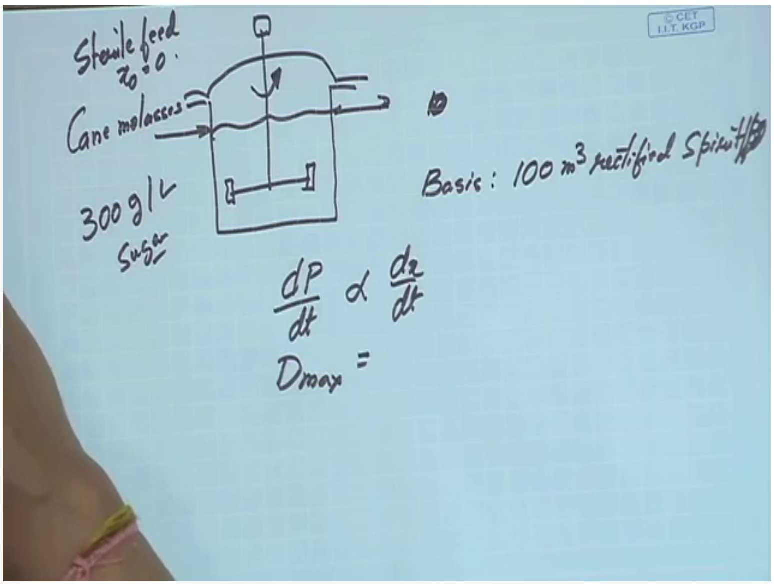

So I can schematically I can write this process like this, it is like this so we shall have to produce that here we are targeting basis because every, what is the basis? Basis is 100 cubic metre rectified spirit. Let us assume per day or you know they rectify spirit not per day rectified spirit that is the that is the production that we have and let us assume this is per day is the production we can we can elaborate that I think that we can per day.

So and which contain 90 per cent of volume by volume of ethanol, so here we put the cane molasses which contain about 300 gram per litre of sugar, am I right? So you and then we operate this we have we have this equation X0 value here we assume this X0 value here is 0 because we assume this is sterile feed, sterile feed we can assume then what will happen that this is rotating like this then we can find out under steady state conditions where first you can find out what is the at what dilution rate we can get the maximum cell mass production because we observed that rate of product formation is proportional to rate of cell mass growth because we already pointed out the ethanol fermentation is a growth associated product.

So since is a growth associated product so we assume the if there is a maximum of cell mass growth that will give the maximum amount of product formation so we try to find out that at the under what circumstances it produce the maximum amount of cell mass.

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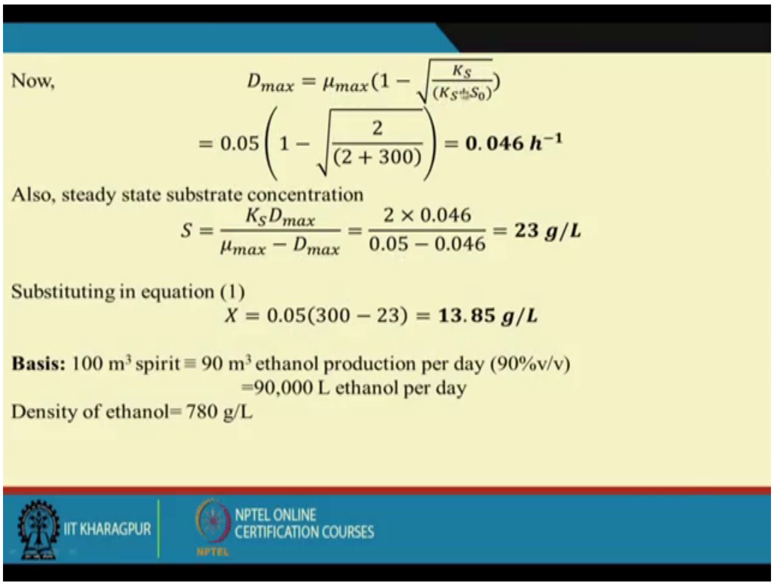

We calculate the Dmax value, Dmax value is equal to Mu max it is it is mentioned here Mu max 1 minus into bar Ks by Ks plus S0 then we have all the values here, so we can find out the Dmax value is 0.046 hours inverse.

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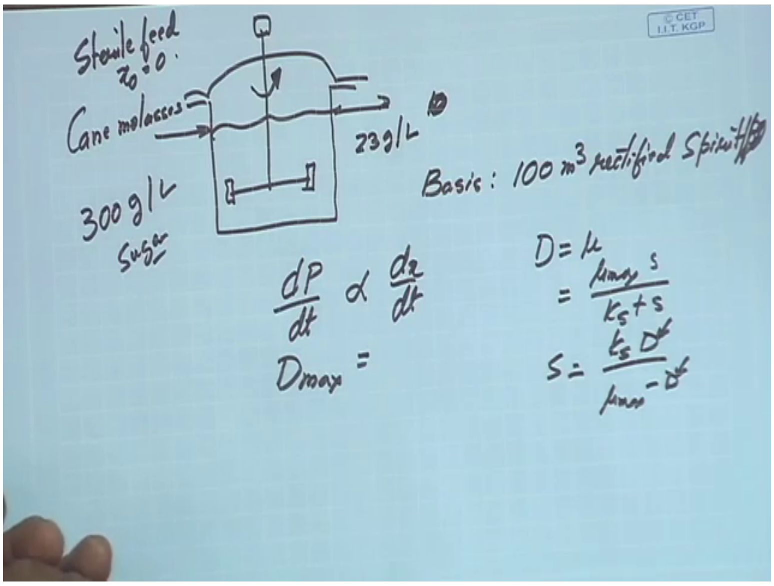

Now under steady state conditions we already find out that under steady state condition and sterile feed we find D equal to Mu so this is equal to Mu max S Ks plus S and from that we can easily find out the value of S, S is coming as Ks D Mu max minus D, now this D we can replace by Dmax and we can find out the value of S. So S here S is coming about 23 gram per litre, so here it was 300 gram per litre is coming like this though by substituting we can find out the cell mass concentration.

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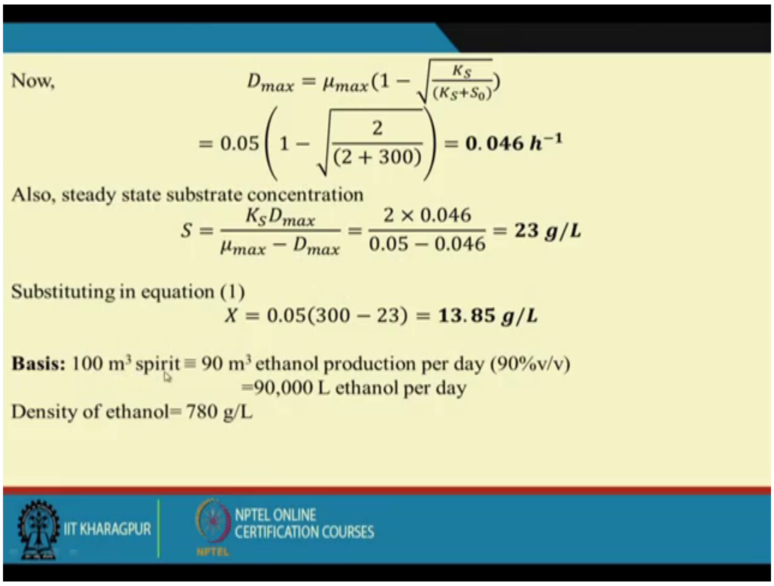

Now 100 cubic metre spirit that means 90 per cent volume by volume that means I can assume it has 90 cubic metre ethanol is to be produced per day and 90 metre cube ethanol is equivalent to 90,000 litre ethanol per day. Now density of ethanol pure ethanol is 780 grams per litre.

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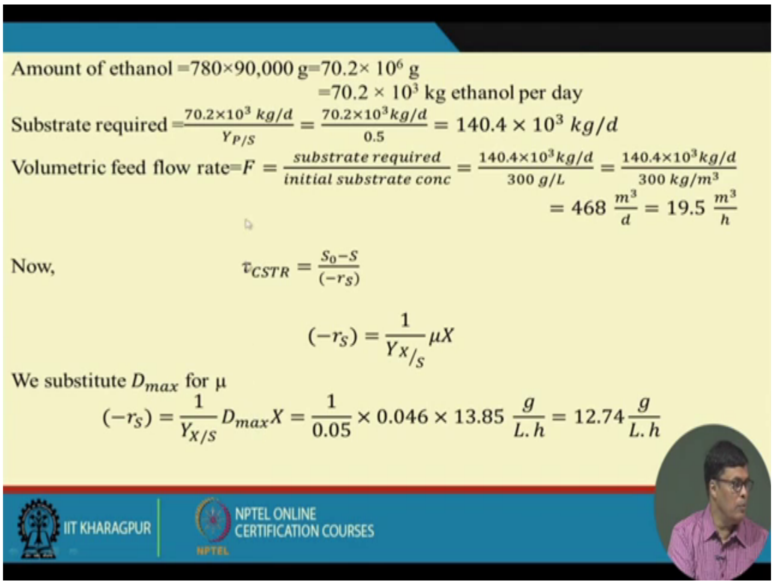

Now if it is like this then we can easily calculate how much ethanol is produced this is about 70.2 into 10 to power 3 kg ethanol per day. Now then we can we can easily find out the substrate required is how much this is 140 because the Yp by S ratio that is gram ethanol per gram of sugar is this so you can easily calculate that you know that what is the how much sugar is required per day. Then volumetric flow rate we can find out because this is the substrate required divide by that initial substrate concentration we can find out the what is the flow rate.

Now since this is the CSTR we can easily find out tau CSTR the space time that required for the continuous start time reactor is the s0 minus S minus rs this already we derived in the our previous lectures then minus rs I can write.

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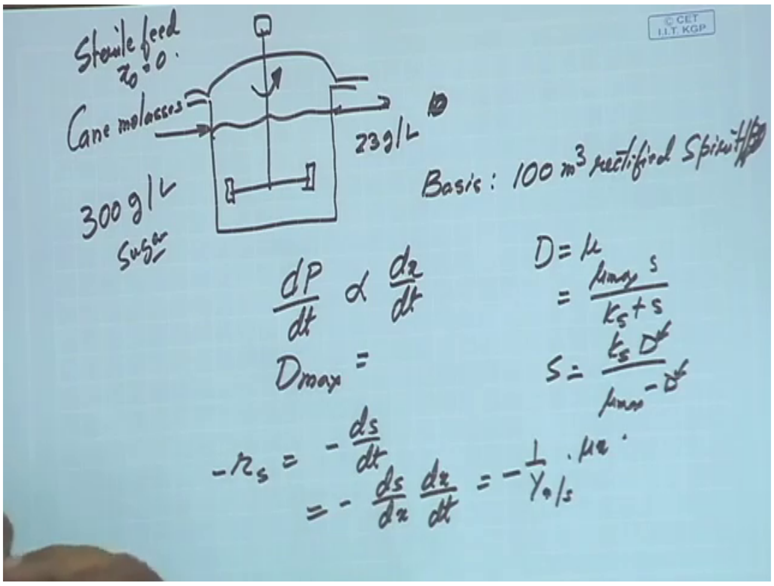

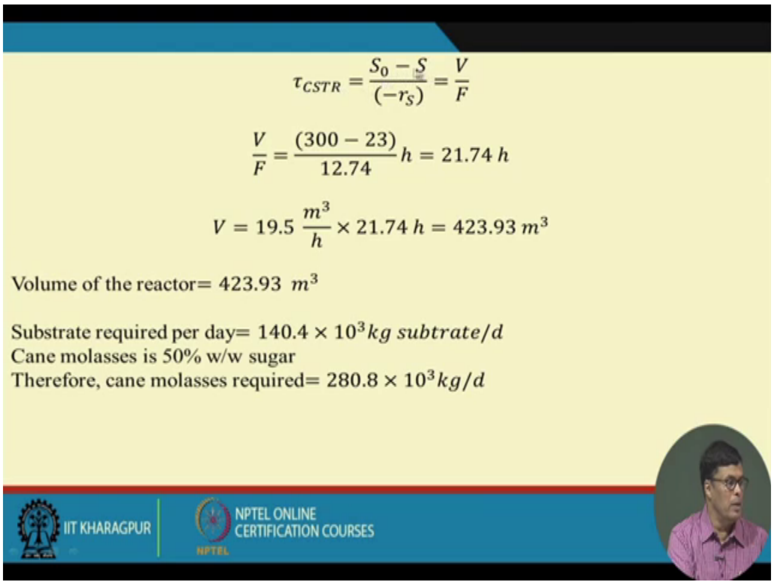

This is how we can write the minus rs because minus rs is equal to minus ds by dt, the minus ds by dt I can write ds by dx and dx by dt. This is minus this is equal to Yx by s into this is Mu into x this is exactly what is written here and then we can calculate the value of minus rs and once you calculate the minus rs then we put the value in the tau CSTR this is S0 minus S minus rs we can easily calculate the what is the value of tau CSTR.

Now tau CSTR is coming at 21.74 hour and then this is equal to V by F, V by F is the this is equal to the that volume by volumetric flow rate volumetric flow rate already we have calculated and in the previous we have calculated the volumetric flow rate is 19.5 metre per hour. So here we can we can put these values we can multiply this with tau CSTR we can get the volume of the reactor.

The volume of the reactor is coming like this substrate required is this and cane molasses contained about 50 per cent of sugar so you divide by 0.5 it will come in 280.8 into 10 to power 3 kg per day. So this much of cane molasses is required for the for the getting 100 cubic metre of rectified spirit. So we can easily calculate is not very difficult we can calculate the how that whatever amount of ethanol produced by the industry we can easily calculate.

And since now-a-days most of the industry they are going for CSTR process because which has the higher productivity so this kind of analysis always will be helpful. Thank you very much.