Aspects of Biochemical Engineering
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Lecture – 38
Kinetics of Substrate Utilization, Product Formation and Biomass Production of Microbial Cells – VIII

Let me welcome you back to my course aspects of biochemical engineering. Now in the last lecture you can remember I try to discuss some a numerical problems and I analyze the; try to analyze the batch and fed batch process. So, we can we have just to find out that a required time we can get that you know a maximum cell mass concentration in a batch process, and also in the fed batch process we can find out that how much maximum cell mass we can produce, and what is the time required for the fed batch operation all this thing we try to analyze in the last lecture.

Now, this lecture is little bit different, because we will be again discussing with the different numerical problems, but mostly we will be concentrating on the chemostat process. Now chemostat now process is considered as the CSTR. CSTR is the continuous start and reactor as you know when you use that in the biological system, we call it chemostat. Now chemostat at the kind of process which can be very easy to operate, and not only that productivity of the chemostat process is a much high as compared to batch process only; only I told you the problem is the contamination we have in the during the long time operation otherwise this is the process through which we can get the maximum amount of product formation.

So, let us start with because we will be taking the example of the different fermentation process as you know the saccharomyces cerevisiae can be used both for baker's yeast production as well as of ethanol production both the fermentation process will be dealing with and plus we want to discuss some other numerical problems. Now first let us start with that the baker's yeast fermentation process.

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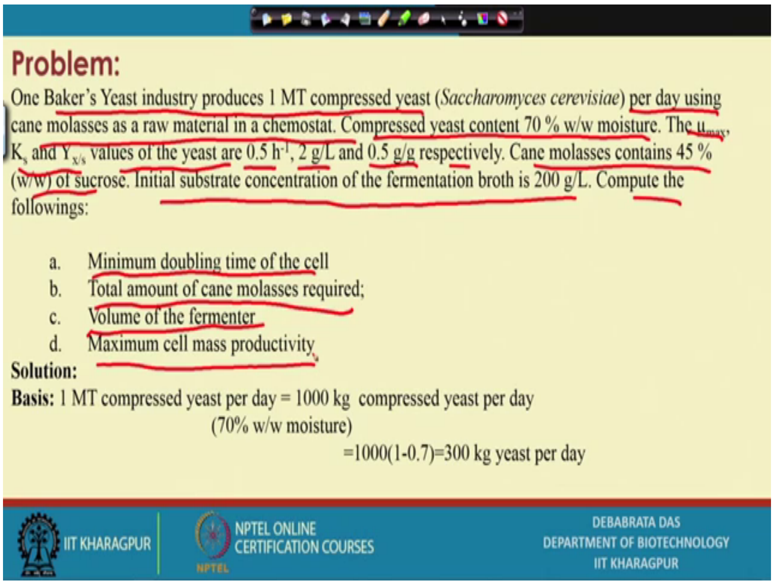

Now, if you look at their baker's yeast a one the problem is one baker's yeast industry produces one metric turned of compressed yeast now, what do you mean by compressed yeast? Compressed yeast means that as you know that the baker's yeast the industry, they usually produce two type of yeast one is called compressed yeast, another called the active dry yeast. Compressed yeast content more than the 70 percent moisture.

But in the active dry yeast content about 6 percent moisture. So, active dry yeast whenever we produce this is the used for longer time of operation and compressed yeast whenever we produce that is for very short time, because it contains 70 percent of moisture. Since the moisture content is very high. So, there is the every possibility of contamination, because the any bacterial cell can grow because this is it contains we know that yeast content about 50 percent of protein and also it contains vitamin b. So, you any bacteria can grow very easily and the each cell.

So, this is this the compressed yeast usually to be marketed a 100 the refrigerated conditions the this problem deals on the basis of that now, that a one metric tons compresses that and per day using the cane molasses as the raw materials in a chemostat. Compressed yeast content about seventy percent weight by weight moisture, mu max K S and Y x by S value are given 0.5 hour inverse to 2 gram per liter of K S and Yx by S is 0.5 gram per liter respectively.

Cane molasses contains about 45 percent weight by weight sucrose; initial substrate concentration of the fermentation broth is 200 grams per liter compute the followings. What we shall have to compute we shall have to find out what is the minimum doubling time of the cells the total amount of cane molasses required volume of the fermenters and maximum cell mass productivity.

So, this is the I considered this is a very important problem as per have the bio chemical industries constant particularly baker's yeast fermentation is concerned, and this in this particular connection I want to tell you that if you look at the most of the biochemical industry or industry that we have in India that most a industry they usually operated in a batch mode.

But as soon as the world w trade organization treaty was signed then we have open trade in the different country, then all the industry they switch over to the they convert batch to the continuous process. The beauty of the continuous process is that your labor percentage labor consumption will reduce to ten percent and your amount of product formation increases more about 10 folds. So, there is a huge amount of productivity increases. So, this problem is very important. So, when you start with one metric tons of compressed dry yeast per day what do you mean by that.

One metric ton means 1000 kg compressed yeast per day. Now it contains about 70 percent moisture. So, how much a total solid will be there 30 percent. So, naturally 1000 into 0.3 that will be 30 kg dry yeast to this to be produced per day am I right.

Now, first we shall have to find out that what I shall have to find minimum doubling time am I right.

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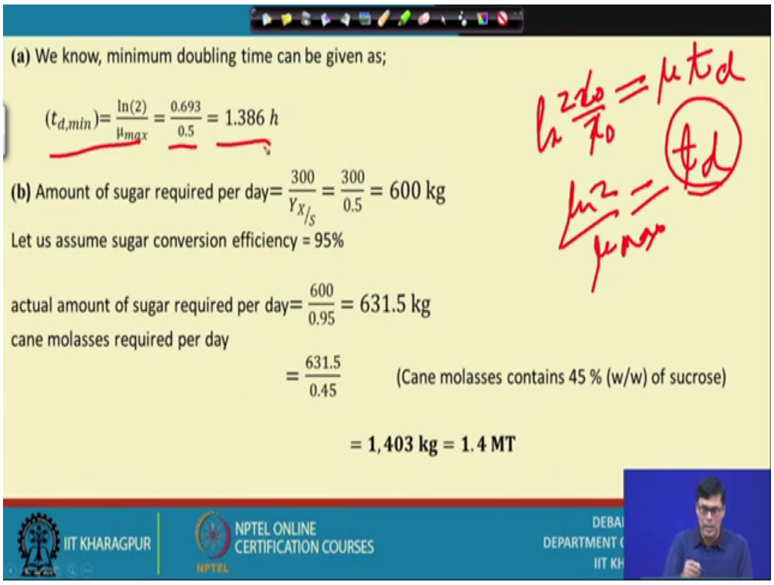

So, what is the minimum doubling time we have we know that we have already shown you that the lnx by x 0 equal to mu into t am I right? Doubling time means doubles the cell mass.

So, this is the doubling time. So, x 0, x 0 will cancel. So, ln 2, ln 2 by mu is the is the is the is the t doubling time. Now when this doubling time will be minimum when mu is mu max then easily minimum this is exactly what we have written here d doubling time because ln 2 by mu max and ln 2 is 0.693 and the mu max is 0.5. So, it is coming around 1.386 hour that is the minimum doubling time that required for the cells.

Now, next problem is that total amount of cane molasses required that we shall have to find out am I right. So, the question come how we can find out. Now in this problem that amount of cane molasses required per day how we can find out.

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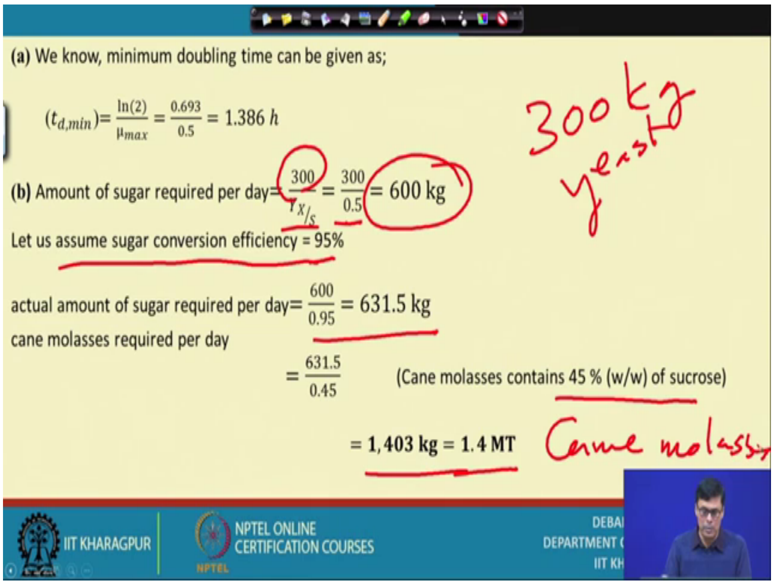

So, 300 we shall have to produce how much? 300 kg of yeast the dry yeast am I right. So, an yield coefficient is 0.5. So, if you divide by that you will find how much substrate is required sugar is required this is 600 kg.

Now, let us assume the sugar conversion efficiency is 95 percent, because it is the this is the theoretical value am I right. So, may be may not be the all sugar will be converted into the cell mass, the let us assume that 95 percent is the conversion efficiency, then actual amount of sugar requirement will be 631.5 kg. Now cane molasses contained about 45 percent of weight by weight of sugar. So, if you divide by 0.45 then we will get one point four metric tons of the cane molasses this is cane molasses, that we will required for this, the which was second part of the problem can be solved very easily.

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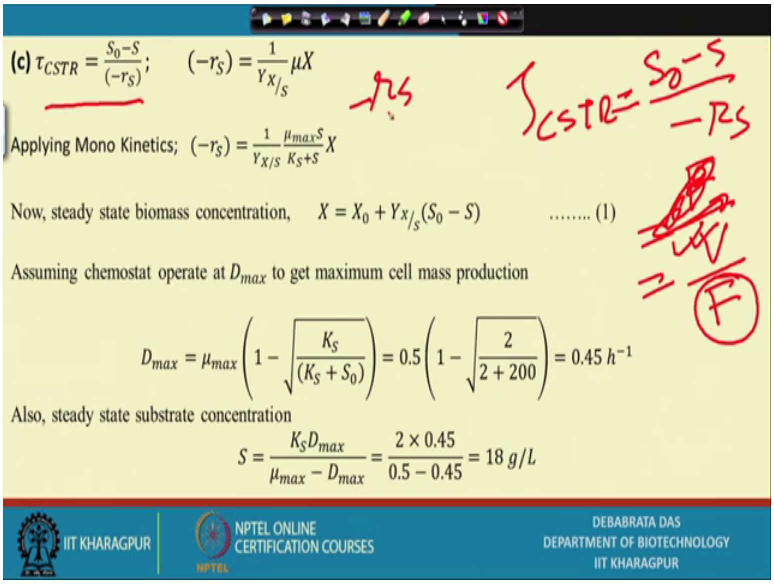

Now, the third problem that we have, that we shall have to find out the volume of the reactor; now how we can find out the volume of the CSTR right I told you, you can remember in CSTR first we shall have to find out tau CSTR what is the tau CSTR? Tau CSTR equal to S 0 minus S divided by minus Rs am I right. Now once you know that then this is equal to F by sorry this is equal to now this is only this is equal to V by F.

Now, if you know the flow rate that volumetric flow rate, then you can find out the volume of the reactor. So, this is how you can find out the volume of the reactor now here exactly that is written here that this equal to the tau CSTR equal to S 0 minus S by minus Rs.

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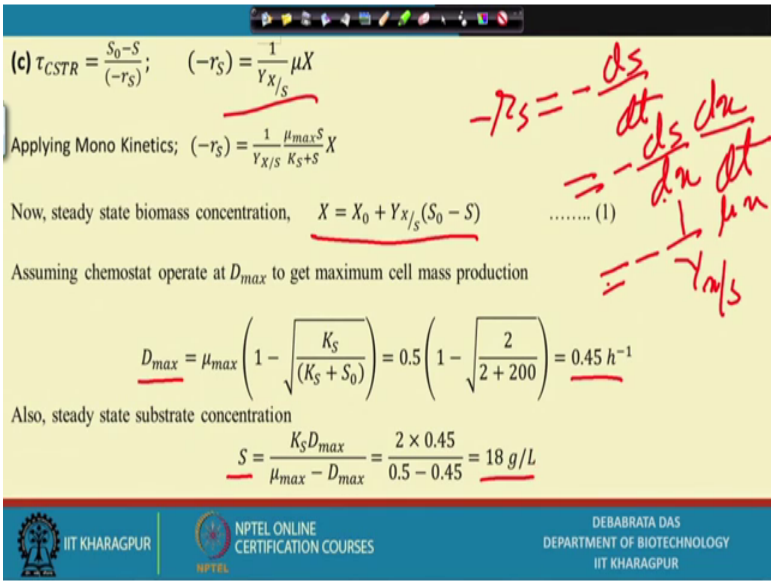

Now minus rs how we can write minus rs; minus rs is equal to what? Minus ds by dt am I right. Now this is equal to minus ds by dx by dx by dt now this is equal to dx by ds 1 by. So, this is 1 by y x by S into this is dx by dt is equal to what? This is equal to mu into x.

This is exactly what we have written here. Now from the Mono Kinetics we know mu equal to mu max S K S plus S and X is like this. Now what is the question come what is the maximum cell productivity? Maximum cell productivity means that we know there is a situation called d max what is d max? D max is the dilution rate when you will get the maximum rate of cell mass production am I right now. So, we shall have to first find out the d max then we shall have to find out that d max, what is the cell mass concentration. So, d into x if you d multiplied with x then you will get the maximum cell mass productivity this is exactly what we are trying to do here.

So, first we shall find out the d max value, and we find this is 0.45 hour inverse, and then corresponding S value we find out this is this is the 18 points S value is K S into D max a mu max minus D max. So, we can find out this is about 18 grams per liter that we have.

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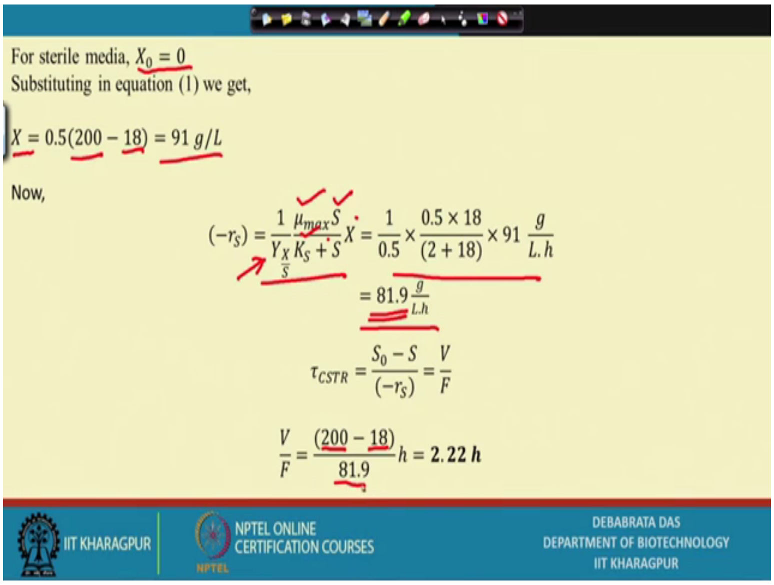

Then if you come to this equation, then we find that the in the sterile feed when you have sterile feed x 0 equal to 0, and then by substituting this the initial substrate concentration was 200 grams per liter, and final substrate concentration was that is steady state substrate concentration 18 grams per liter.

So, what is the X value will be yx by S into 200 minus 18, this is 91 grams per liter. Now what is the rs value? That we have written this is the equation. Now in this equation we can put that the value of a Y X by S mu max then S then K S, S and X we can all the values is available with us and we find out this is 81.9 grams per liter per hour. Now tau CSTR we have find this is the S 0 minus S by minus rs is equal to V by F. Now what is the S 0 value is 200 grams per liter, S is the 18 grams per liter and this rate of substrate degradation is 81.9.

So, this will give the 2.2 hours.

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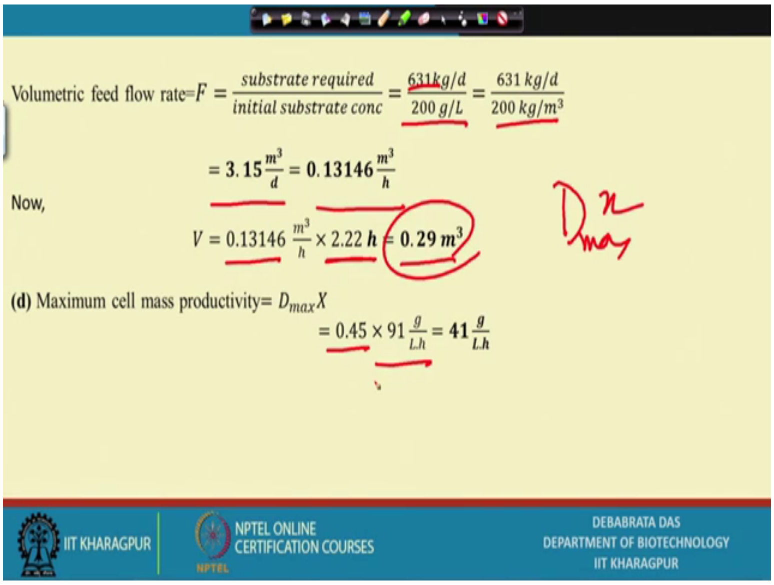

Now, shall have to find out a, you see that if you look at the previous equation this is equal to V by F. So, we shall have to if we want to find out the volume of the reactor, we shall have to find out the flow rate. So, how you can find out the flow rate? Flow rate can be find out what is the substrate required that we have already require calculated the 631 kg per day, and what is the what is the initial substrate concentration is 200 grams per liter. Now 200 grams per liter is equal to 200 kg per cubic meter.

So, this will be converted into this we can write in this form, 3.1 this is cubic meter per day. And this we can convert in terms of the hour by divided by 24. Then the 1.131 cubic meter per hour. Now we have find out that total time required for the CSTR is 2.2 hour. So, you multiplied this flow rate you will get the volume. So, this is the volume of the reactor we can and then I we at the then at the same time I told you that how you can find out the maximum D into x.

Now, what is the D value D max value we calculate 0.45; what is the that and that you know x let me let me see that. Now maximum cell productivity we still have to find out then we that you know x the value we shall have to find out that I think this the x value that we shall have to determine and the x value we find is about 91 grams per liter.

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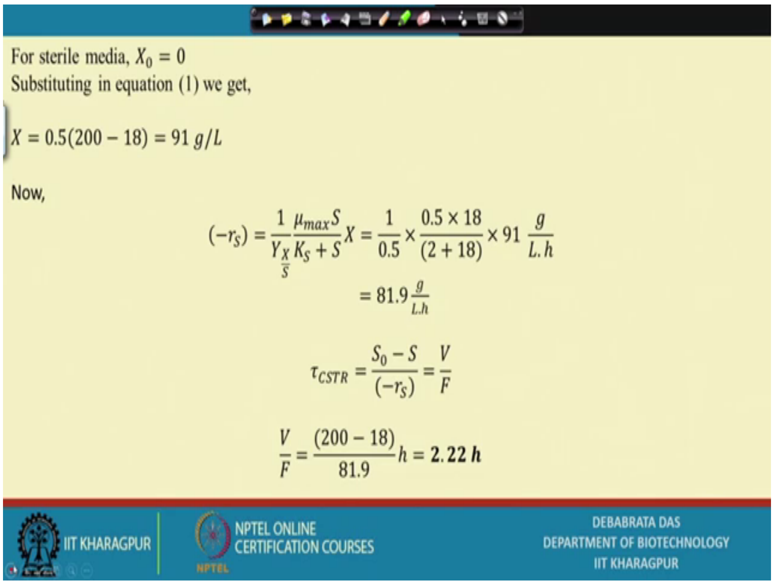

So, we can this even it is our inverse; so it is it will come. So, it will come around 41 gram per liter per hour. So, this is how we can solve this equation.

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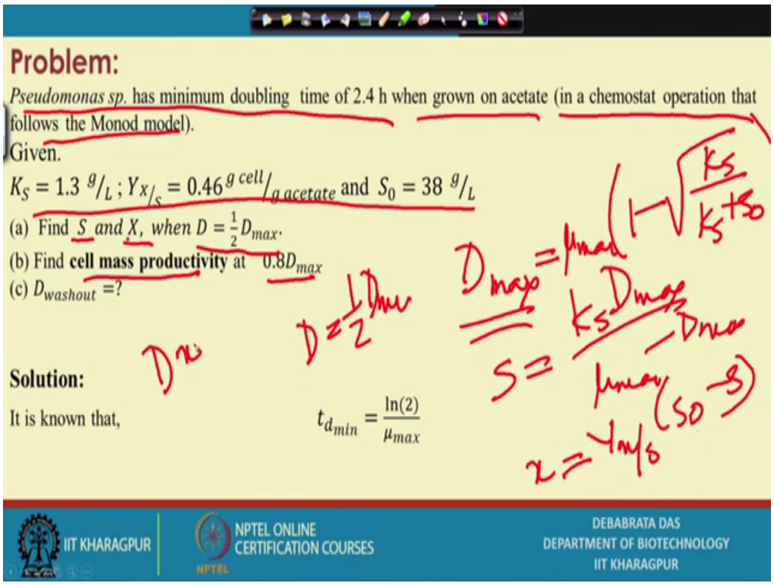

Now, next problem also very interesting that pseudomonas spaces I, as pseudomonas does not require any kind of introduction, because pseudomonas is the first organism genetically modified organism that was patented in the USA, and one of the Indian scientists they might be knowing (Refer Time: 15:11) Chakravarthy, they he bring out the patent and this pseudomonas is very much famous for is considered as the oil eating bacteria; because lot of a oil spillage we have in the ocean and this bacteria can be remove the that oil from the sea.

So, this problem deals with that that the pseudomonas basis is the minimum doubling time is the grown on the acetate, and in a chemostat operation the Monod and these are the different value very simple thing. What we want to know that, what will be the value of S and X; S and X at the dilution rate d equal to half D max. So, we can first we can easily find out what is the D max value, what is the D max value this will be equal to mu max 1 minus root over K S by K S plus S 0 am I right. So, we can we can easily find out the value of.

So, once we know that we can find out the value of S S is what K S into D max minus mu max minus D max am I right. So, we can find out S value and once you know the x value we can find out the x value x value is a what? Yx by S into S 0 minus s. So, we can find out that the x and S value and d max is where now d max when d max is equal D equal to half of half of where D max then you can find out also the value of S and X and once you know that then we can find out the what is the value of 0.8 d max then you can find out the cell mass productivity, what is the cell mass productivity D into x and what is the cell washout?

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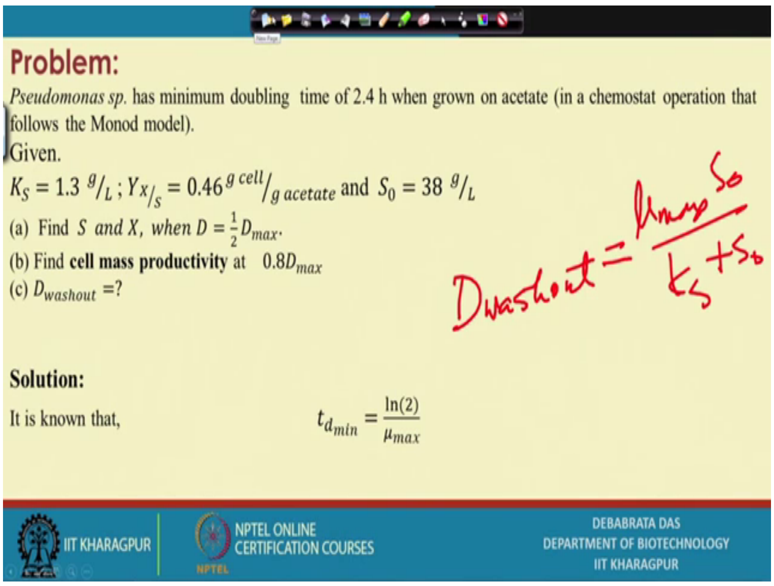

Cell a d washout will be the at washout there is no cell present at the reactor then S will be S 0, then with the equation will be mu max S 0 K S plus S o. So, I hope it is very simple problem I hope everybody can understand it. So, what we have written here the minimum doubling time is can be written as the ln 2 by mu max.

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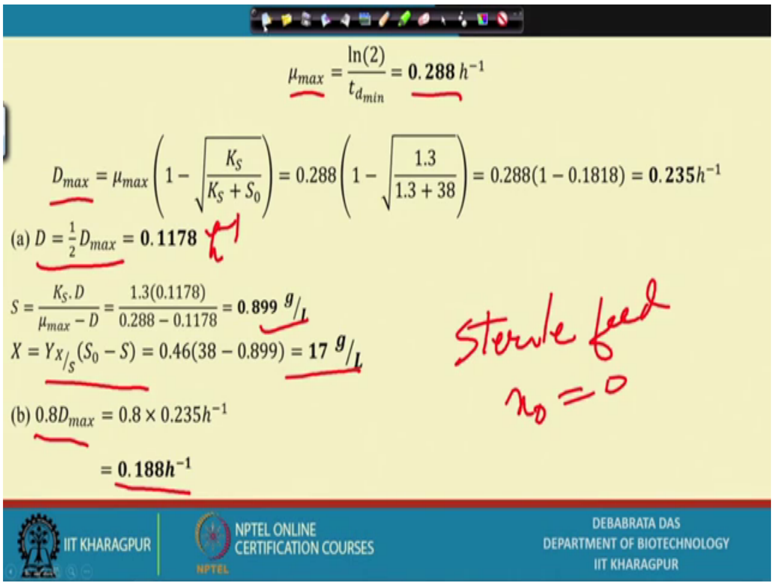

And then we can find out that what is the mu max value is the about this, and once you know the mu max value we can find out the d max value. Once we have D max value we can find out the D value equal to half D max, this is the 0.2 this is the hour inverse am I right.

And then S value we can find out K S D mu max minus this D is nothing, but half D max. So, you know that we can put this value we can find out x equal to this and x equal to this when we consider this sterile fit if you have the sterile fit, then what will happen that the x 0 equal to 0 am I right and then we can have this equation we can put it this is 70 grams per liter. Now point when a D equal to 0.8 D max, then we can this D value will be 0.188 hour inverse.

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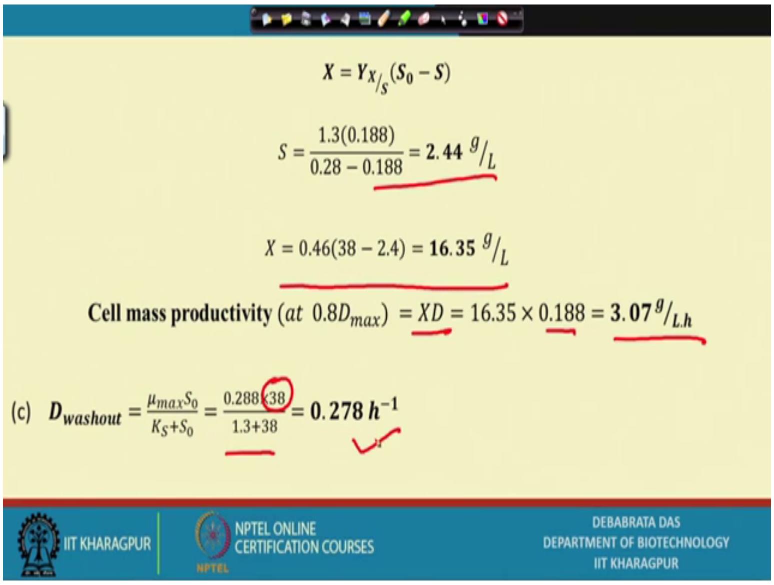

Now, Y X by S, Y X will be x equal to what Y X by S 0 minus S. So, we can easily find out the respective value is of steady state substrate concentration and steady state cell mass concentration we can find out. Now what is the productivity? Productivity is nothing, but X into D. So, we know the value of X we know the value of D. So, you multiply that we will get the 3.7 gram per liter per hour. Now D washout I told you that the initial substrate concentration is 38 grams per liter, if you put this value then we will get the that the D washout value.

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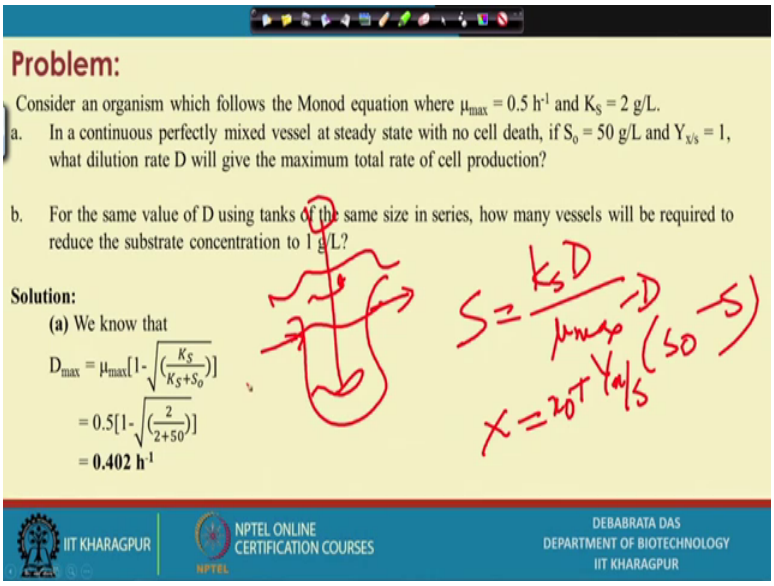

So, this problem is the typical problem, I hope everybody can understand can solve by them by yourself. Now next problem is a very interesting problem that is how we can or we can analyze the casket type of a reactor casket means that multiple CSTR that one after another. If you will do that because why we are interested?

Because the reason is that when we operate a single chemostat, then you know that our problem is little bit simplified because this is single chemostat that would this is coming and going, then we have S value we know S value equal to what K S D mu max minus D am I right and y X value also you can you can easily you find out x 0 plus Y x by S S 0 minus S.

So, you can easily find out, but what will happen when this is connected with the another CSTR suppose you know that.

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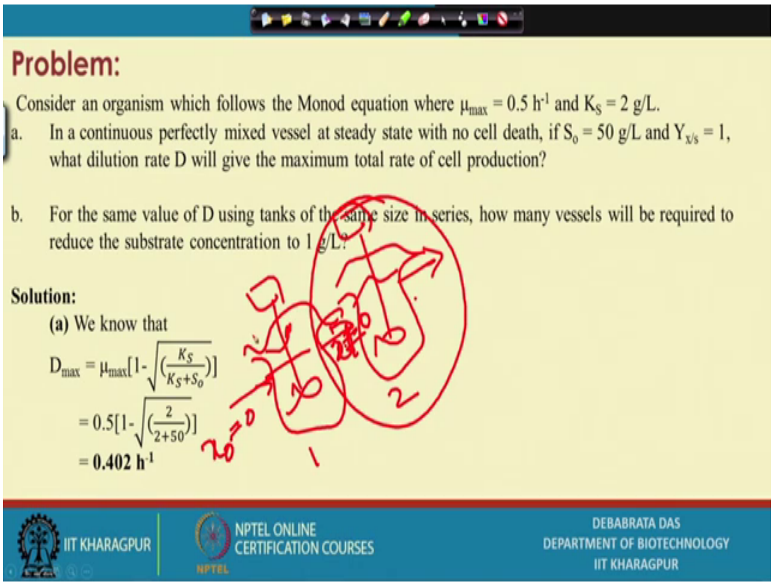

Suppose this is the CSTR, and this is one reactor again it is connected with the another reactor. So, how you can analyze this? Now this is the only the difference is that the when we analyze the one reactor, we assume x 0 equal to 0 am I right, but when we when we go for the second reactor this is first and second here x is not equal to its not equal to 0. So, what you have to do? You have to write the cell mass balance in this equation, and then and only then you can solve this only this problem deals with this I hope you will understand that.

Let us see, how we can done that? Now the problem is that consider an organism which follow the Monod equation and mu max value this, and K S is point the two gram per liter in a continuously perfect mixed vessel at steady state no cell death S 0 equal to 50 grams per liter, Y x by s is 1 when dilution rate d will give the maximum total rate of cell mass production. So, what dilution rate Ds. So, that equation already we know that d max equal to mu max 1 minus root over K S plus S 0, we put the different value we can find out the D max.

So, D max we do not have any problem, because you can do it solve it very easily. Now question come is the second part for the same value of D using tanks of the same size in series, how many vessels will be required to reduce the substrate concentration to one gram per liter; that means, what is says that you have multiple number of tanks am I right.

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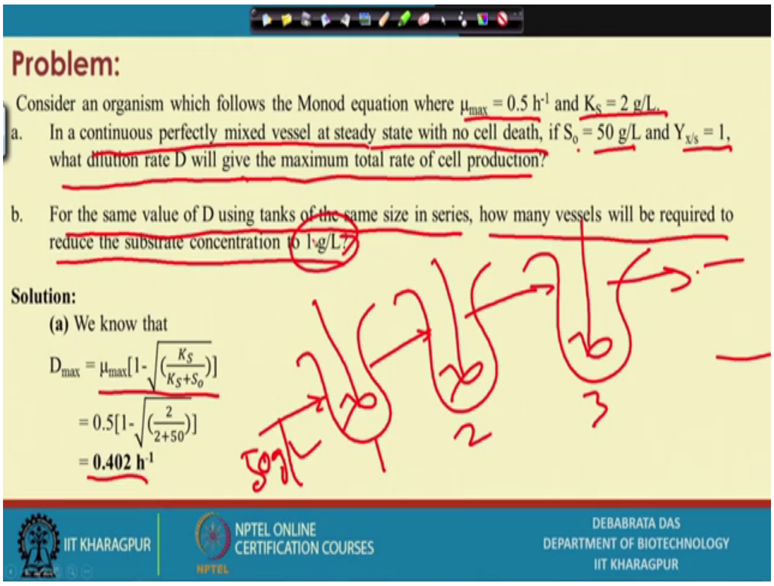

So, this is connected like this, always starting all are continuously its will start and react like this. So, this is the number of reactors. So, here we have 50 grams per liter.

So, problem is that this is 1, 2, 3, 4, 5, 6 now how many is a called that the reactor will be required to reduce the substrate concentration to one gram per liter. This is the problem that we have. Now let us see how we can solve this problem.

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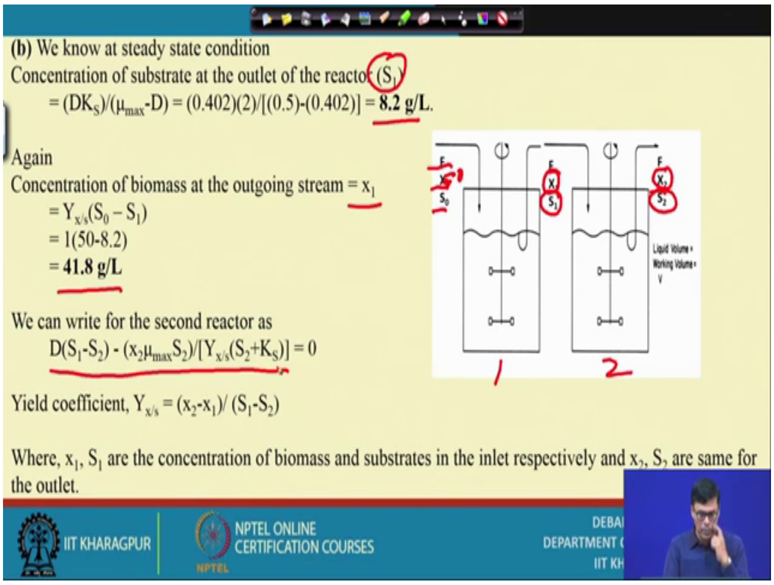

Now, first we shall what we shall have to find out, that we shall have to find out that what is that this is like this is schematically this process is like this is the initially F is the flow rate then X 0 is the initial. So, this we can assume to be 0 in the first reactor am I right x 0 equal to 0.

But the then we can have this equation, we can find out the value of X 1 we can find out the value of S 1. So, S 1 value if we can write D K S minus d divided by mu max minus D. So, we can find out 8.2 grams per liter. Now we can find out X 1 value Y x by S equal to this is 41.8 grams per liter, then first reactor we do not have any problem. The second reactor we shall have to find out that what is the value of x and then we shall we are interested to find out the value of S 2, because then if it is if more than one gram per liter we shall have to analyze again for the third reactor.

So, let us see that in the second reactor what should be the substrate concentration, for doing so, what we can do? We can write the substrate balance and if that the write the substrate balance, this equation will come this is equal to let me let me show you this how this equation has come.

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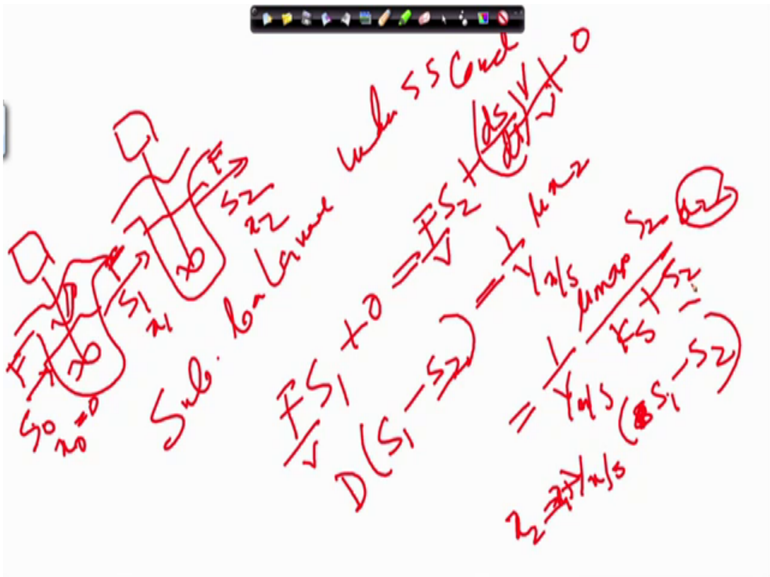

Suppose, what I was telling this is first reactor, this is second reactor am I right. So, this is like this. Now here we have x 0 S 0 this is the S 1 and this is the S 2 am I right and this is the x 0, x 0 equal to 0 and this is x 1 this is x 2.

Now, if you want to do here the substrate balance; under steady state condition what you can write what is the rate of input of substrate? This is the F is the flow rate here, F is the flow rate all the flow rate is F. So, F into S 1 is there in what is the generation of the substrate will be 0, what is the rate of output this is equal to f into S 0 S 2 am I right and what is the rate of substrate consumption it is ds by dt into x sorry this is ds by dt into v this will be v and rate of accumulation that will be equal to 0 am I right.

So, this is the equation that if you divide by v both the side, this is v, this is v, this is v then what we can write this is equal to D is D into S 1 minus S 2 what we can write? This is equal to ds by dx dx by dt that we have shown you before this is equal to Y x by S, and this is mu dx by dt equal to mu into x and then can write this is 1 Y x by S this is mu this will be x 2 second reactor am I right then this is the mu max into S 2 plus K S plus S 2 am I right.

So, this is into x 2 and what is the value of x 2? X 2 will be equal to this is Y x by S x 1 plus Yx by S into S is a S 1 minus S 2. So, we can you can put this value here and then the there will be a quadratic equation only S 2 will be unknown single equation single unknown we can easily solve it. So, in this problem this is exactly what do you have written here.

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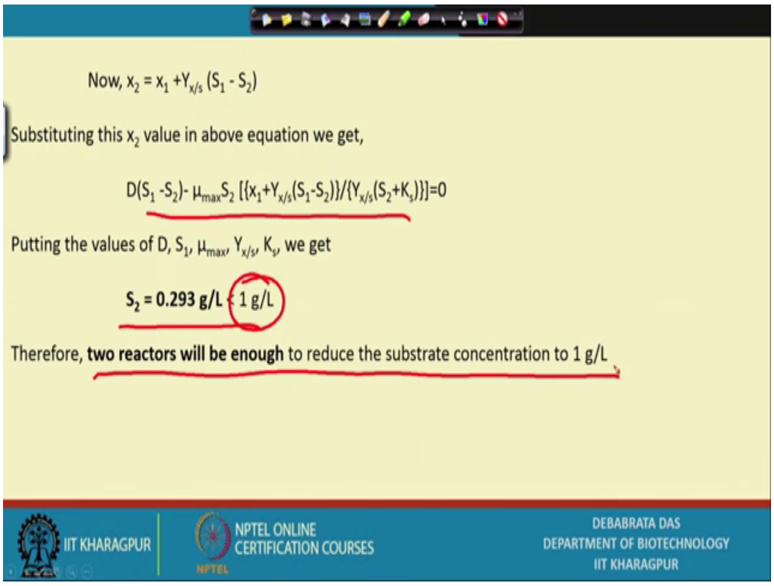

And then we can put this equation, this is the equation as we will build up and finally, we can find out the substrate concentration.

Now, this is coming about 0.293 grams per liter, that mean is a less than 1 gram per liter. So, our course our answer is 2 reactor will be enough to reduce the substrate concentration to 1 gram per liter.

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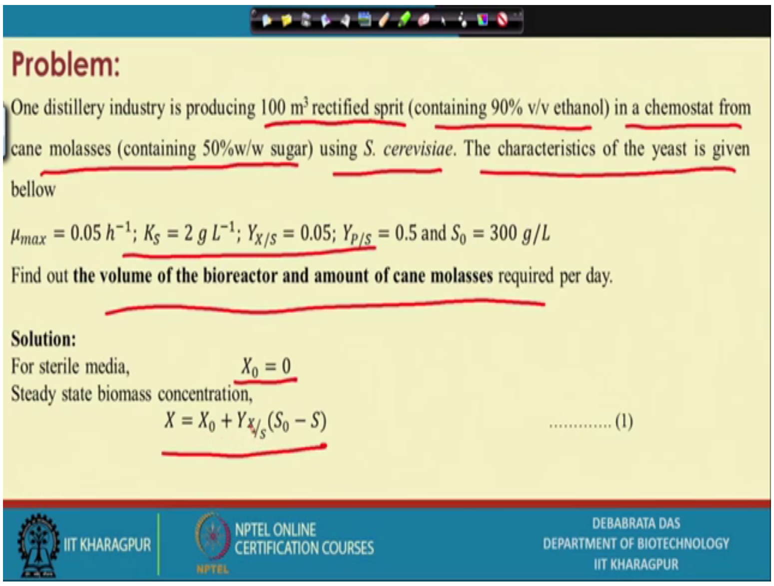

The last problem that we have with the distillery industry, one distillery industry is producing 100 cubic meter of rectified spirit containing 90 percent volume by volume of ethanol in a chemostat from cane molas, containing 50 percent weight by weight sugar using saccharomyces cerevisiae the characteristics of the yeast is given here you have to find out the volume of the bioreactor and amount of cane molasses is required.

They almost similar to the first problem only the here the product is different here product is ethanol. So, here X 0 we can if you consider sterile media, X 0 equal to 0 then steady stress cell mass concentration will be x equal to X 0 Y x by s, S 0 minus S.

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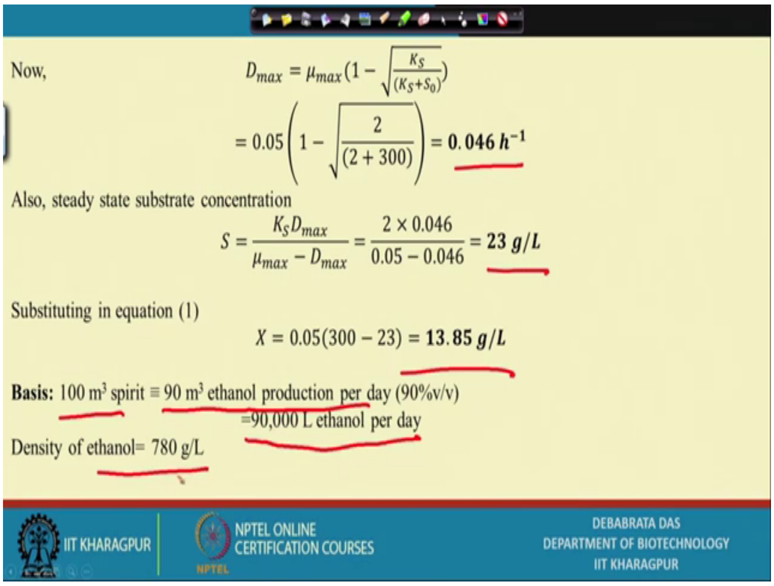

D max equal to this value am I right and ethanol is considered as the growth associated products. So, we assume if we operate in the d max will get the maximum amount of ethanol production.

So, this S value is this and this is the cell mass concentration now hundred cubic meter of spirit, we shall have to produce which content ninety percent of ethanol. So, this will be ninety cubic meter of ethanol 90 cubic meter ethanol, with ninety 1000 liters per day the ethanol density is 780 grams per grams per liter.

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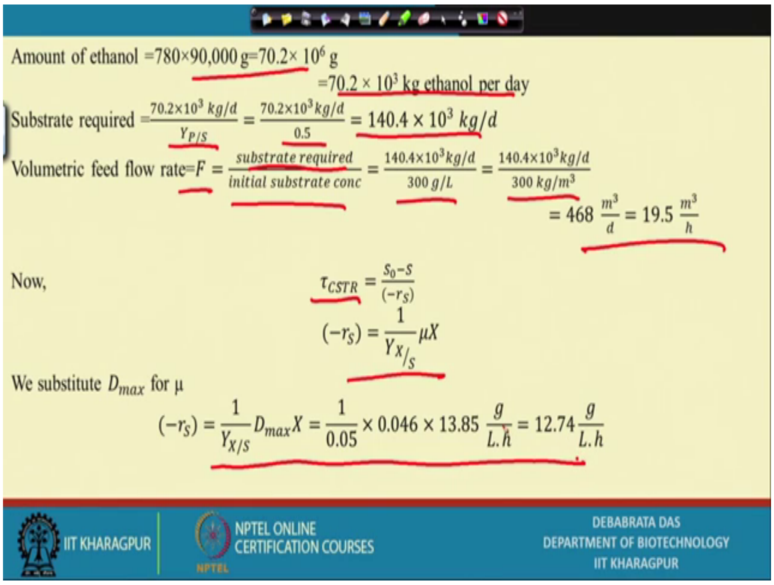

Then we find out what is the total amount of ethanol is to be produced. This is the total amount ethanol is to be produced per day and if we know Y p by S that is the yield of this of your how much product ethanol is produced per gram of a substrates, and we can find out how much sugar is required.

Now, what is the flow rate? Flow rate equal to amount of substrate required divided by initial substrate concentration. So, we can easily find out this is the flow rate, and once if you know the flow rate then you come to tau CSTR equal to S 0 minus S minus rs, rs equal to this rs value we can easily calculated we find out this is the rs value similar to the first problem, that I have I explained I am not explaining again here and we find out the value of tau CSTR is equal to V by S 0 minus S by minus rs equal to V by F.

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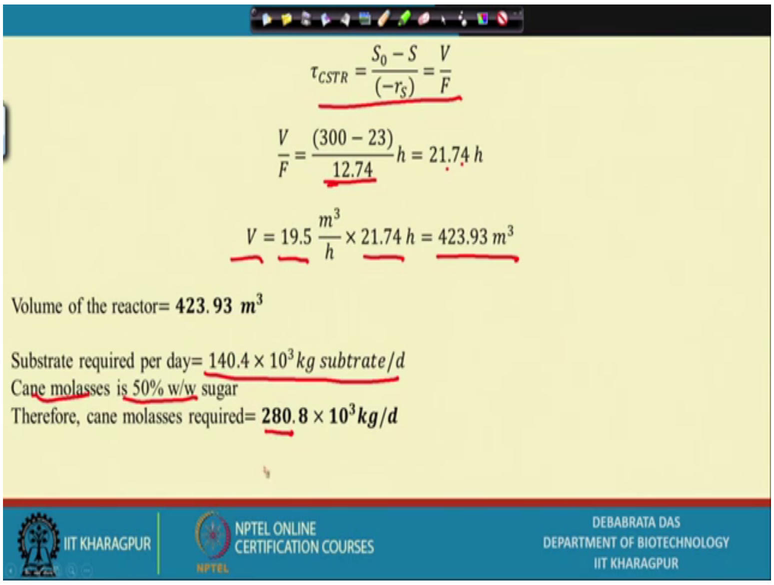

So, we know the this value this is a 300 minus 23 by that a rs is 12.74 and this is equal to 21.74 hours. So, V equal to what will be that this is the flow rate, that we have this is the hour. So, the volume of the reactor will be 423 points 93 cubic meter. Now substrate required per day. So, how you can calculate we can calculate that you know that how much substrate we required per day, we can easily calculate this is this is already we have found out this much of substrate required.

Now, it contains about 50 percent of sugar cane molasses contain 50 percent. So, you multiplied by 2 you will this we multiplied by 2, we will get the amount of cane molasses required for this distillery industry. So, what I conclude here that you know that chemostat process, we can analyze very easily and it is a very advanced process and we try to explain that in a chemostat, how we can get the maximum cell mass productivity, how we can get the maximum ethanol productivity how we can find out the had different dilution rate, how we can find out the substrate concentration and cell mass concentration also I try to analyze the cascade reactor, if you use that multiple chemostat how we can analyze the multiple chemostat process.

Thank you very much.