Aspects of Biochemical Engineering
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Lecture - 30
Kinetics of enzyme catalyzed reactions using immobilized enzymes – II

Welcome back to my course, Aspects of Biochemical Engineering. Now, in the last lecture, I try to explain that the heterogeneous reaction kinetics. And I told you when we immobilize the enzyme on the solid matrix and since your substrate remain in the liquid phase. So, it is a example of the heterogeneous system. And the heterogeneous system, the major problem that we face that is the diffusion problem. And also we have, we have reactions then substrate has to defuse from the bulk to the surface of the solid matrix then and only then it will react with the enzyme and gives the product. And product has to defuse from the substrate, this on the surface of the solid matrix to the bulk of the liquid. So, you know that that diffusion problem is there.

So, there are 2 type of diffusion we come across came across. One is call external mass transfer problem; another is a internal mass transfer problem. Now, external mass transfer problem means, when you assume the enzymes are immobilized on the surface of the solid matrix. So, it is not going inside the solid matrix. So, just at the surface of the solid matrix, the how we can explain that particular situation?

And we have come across the steady state condition where rate of mass transfer equal to rate of reactions and we try to find out the correlation between Damkolher number and effectiveness factor. Damkolher number, I told you it is the ratio between the maximum velocity of reaction divided by maximum rate of mass transfer. And the effectiveness factor is the rate of reaction, the actual rate of reaction divided by rate of reaction when there is no mass transfer limitation. So, then in case of inter particular diffusion problem that we have come across that you know, when the particle present inside the pour, how that can be explained we come across the effectiveness factor and that Thiele modulus and how we try to find out on of what circumstances again it follows it tends to the homogenous reaction.

When eta value equal to 0 that is of that is effectiveness factor is 0, how Thiele modulus that plays important role in the intermolecular diffusion problem. Now this particular

lecture, we try to solve some numerical problems and to have the better idea on the immobilized enzyme system.

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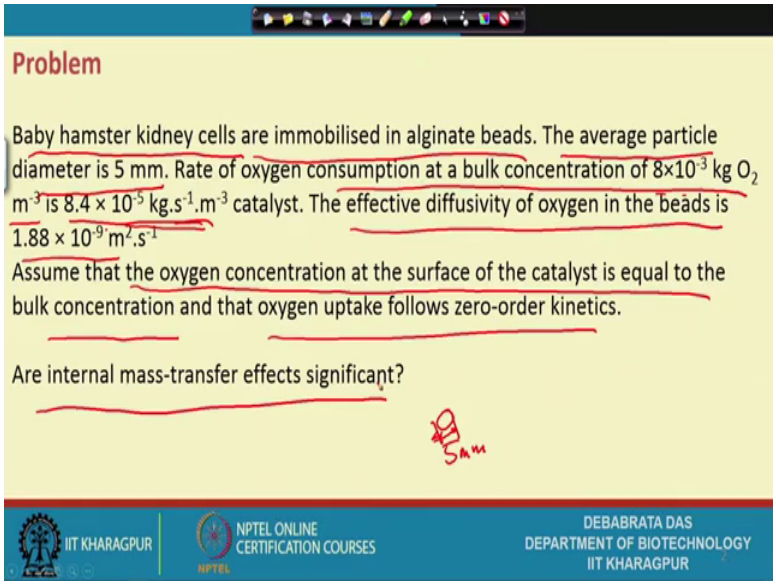

The first thing that I want to show you that this first problem is the; baby hamster kidney cell was immobilized in alginate bead. I told you the alginate bead that in the previous lectures, the how the alginate bead formation take place? We know the sodium alginate that is the soluble material. Now we what we can do, we can this cell we can make a suspension in the in the that sodium alginate, then drop by drop if you put in the calcium chloride solution, then alginate bead formation is there then what will happen your cells will entrap inside the solid matrix that you can you can have.

So, this is exactly what here the baby hamster kidney cell are immobilized in the alginate bead. The average particle diameter is 5 millimeter. The particle diameter means the size of the particle this is equal to what you call this is equal to 5 millimeter ok. The rate of oxygen consumption at the bulk concentration, this is 8 into 10 to the power minus 3 kg per oxygen per cubic meter is this, this is the bulk concentration and the this is the consumption rate this is per unit time what is the consumption rate. The effective diffusivity of oxygen in the bead is 1.8 into 10 to the power minus 9 meter square per second.

Assume that oxygen concentration at the surface of the catalyst is equal to the bulk concentration the oxygen uptake follows the 0 order kinetics. So, then you have to find

out are the internal mass transfer effects it is significant. So, this is the purely a mass transfer related problem and this is the heterogeneous system. So, let us see how we can solve it.

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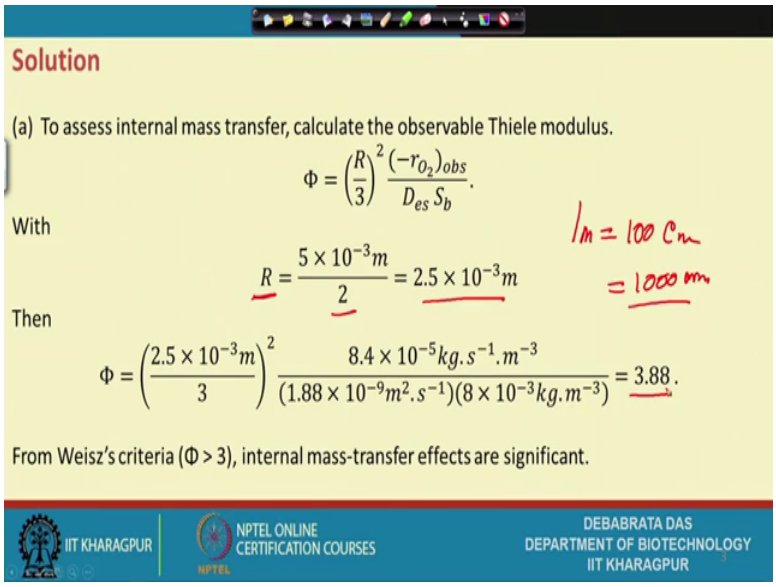

So, to access the internal mass transfer, we have we already know the observable Thiele modulus; this equation we have already seen. This is the Thiele modulus. Thiele modulus equal to R square is the diameter; this is the and by 3 whole square the minus o , that is the here the previously we told about the rate of reaction, this is the rate of oxygen transfer that is the observe and this is the diffusivity and this is the substrate concentration now.

In this in this problem, if you look at the previously that you know how what is R; question is that what is R? What R is, this is the average particle diameter is about 5 millimeter am I right. Now if it is 5 millimeter then, how you can 5 millimeter how you can write. You can write about this is 5 into 10 to the power minus 3 meter. What is meter; 1 meter is how much? 1 meter is equal to 100 centimeter am I right. And what is a millimeter? This is 100, 1000, 1000 millimeter am I right. So, one way that is why we just 10 to the power minus 3. So, this is the radius will be what divided by 2. So, it has come like this. Now if you put this value this all values are there that is equal to 3.88.

Now what we have observe that in the table that in the last lecture I have shown you that if the Thiele modulus is more than more than more than 3, then it is mass transfer

limitation problem that we have in the in the in the in the system. So, that is exactly we have written form Weisz's criteria that if phi is greater than 3, the internal mass transfer effect is significant. So, this problem is very simple. Only whatever data is there we just put in this equation then try to find out the Thiele modulus and if you find out Thiele modulus from that we can speculate whether how what is the diff if it is less than 3, then we can we can we may think little bit different 0.3 then it may be mass transfer problem will be less than reaction problem will be there, but since it is more than 3 week, it clearly indicate this is this is mass transfer the limitation problem will be significant.

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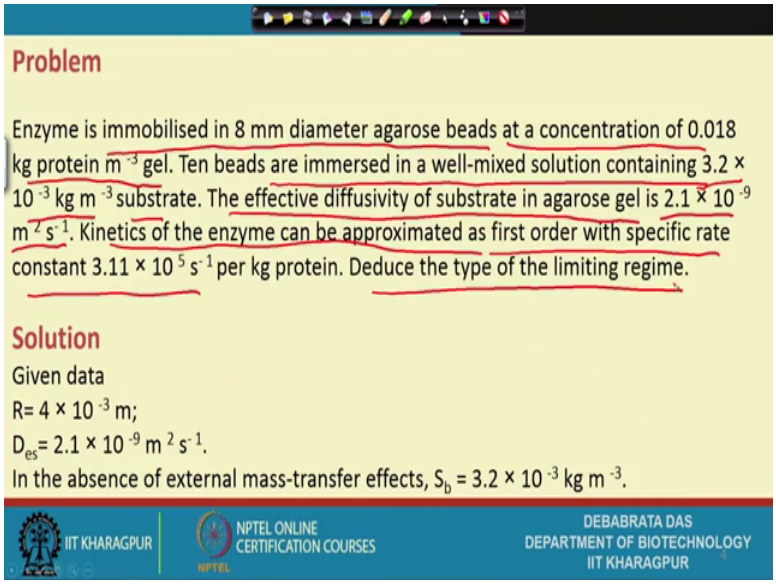

Now, let me go to the second problem. Second problem is what? Second problem is the enzyme is immobilized in 8 millimeter diameter agarose bead and at a concentration of 0.018 kg protein per cubic meter gel. 10 beads are immersed in the well mixed solution containing 3.2 into 10 to the power minus 3 kg per cubic meter of substrate. So, this is the substrate concentration that we have. The effective diffusivity of the substrate in agarose gel is 2.8 into 10 to the power minus 9 square meter per second, the kinetics of the enzyme can be approximated by the first order with specific rate of constant of point 3.11 into 10 to the power 5 second inverse per kg protein.

Deduce the type of limiting regime that whether is the mass transfer limiting regime or it is reaction rate of reaction controlling that we shall have to find out from this particular data that we shall have to calculate. Now what is given here, this is here the R, the size of

the particle is given; this is 8 millimeter am I right? This is the diameter of the bead 8 millimeter means radius will be you can, I showed you before also how it is millimeter can be converted into meter, then it will be 4 into 10 to the power minus 3. What is the diffusivity that we have here; diffusivity is given here, am I right. So, we can write diffusivity in the absence of external mass transfer the S b will be S b equal to S b as the what is the, what is the substrate concentration that we have that this is the substrate 3.2 into 10 to the power minus 3 kg per cubic meter.

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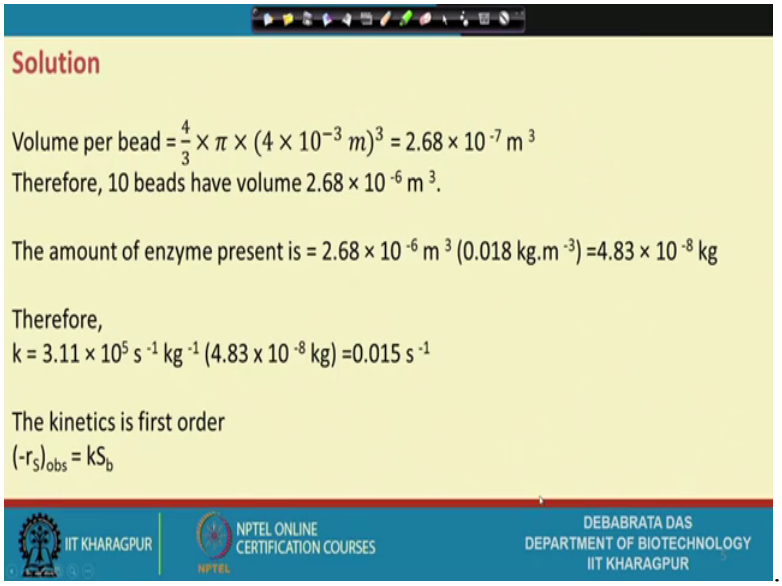

Now, volume of the bead, how we can calculate the volume of the bead. We know the size because what we have already reported the size is 4 into 10 to the radius is 4 into 10 to the power minus 3 meter am I right. Then volume of the is 4 by 3 pi r r cubed. So, this is this is exactly, this is we consider this spherical particle. So, if we consider this spherical particle we can find out easily the volume of this. And there and 10 beads we have taken into consideration. So, if you multiply by 10, it will be 2.68 into 10 to the power minus 6 cubic meter. Now amount of enzyme present is the how much is there, this is this is 2 point this. This is the per cubic meter, this is the concentration of the enzyme.

So, if you multiply that, will get the how much kg of enzyme present; the k is k the rate constant if you look at the rate constant is what that you know that in this kinetics of the approximate the specific rate is 3.11 into 10 to the power 5 per second per kg protein, per

kg protein. So, we calculated the amount of protein in this problem, we calculate the amount of protein. So, you have to multiply this amount of protein that is present there, then we get the k value and k value is coming about that 0.015 second inverse. And what is the kinetics of this, this kinetics this follow the first order kinetics the; this k into S b.

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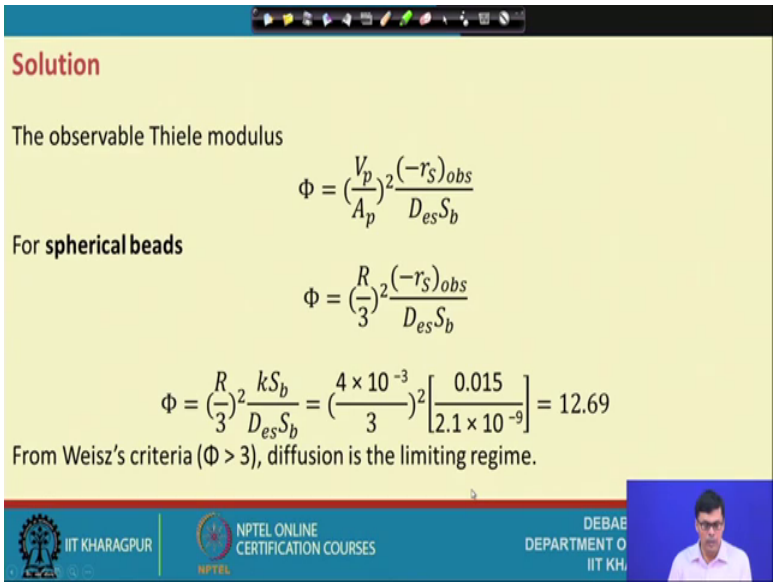

Now, again we know the Thiele modulus is the pi equal to V p by A p square into minus r S observe D e s into S b. Now if you put these values here, the how much values we are getting the 12.69. Now 12.6 the Weisz's criteria that we have if phi value is more than 3, the diffusion limiting regime is there. So, it is very clear that this problem is diffusion controlling because this is not reaction controlling or so, this problem also like in the previous problem we have we can conclude this is also that that mass transfer controlling.

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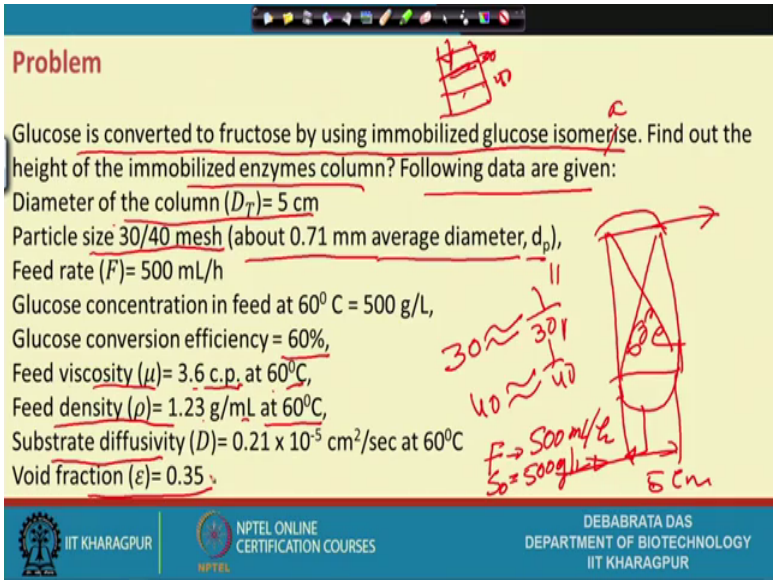

Now, this is the one problem that we have. Now let me explain this problem, the problem is like this.

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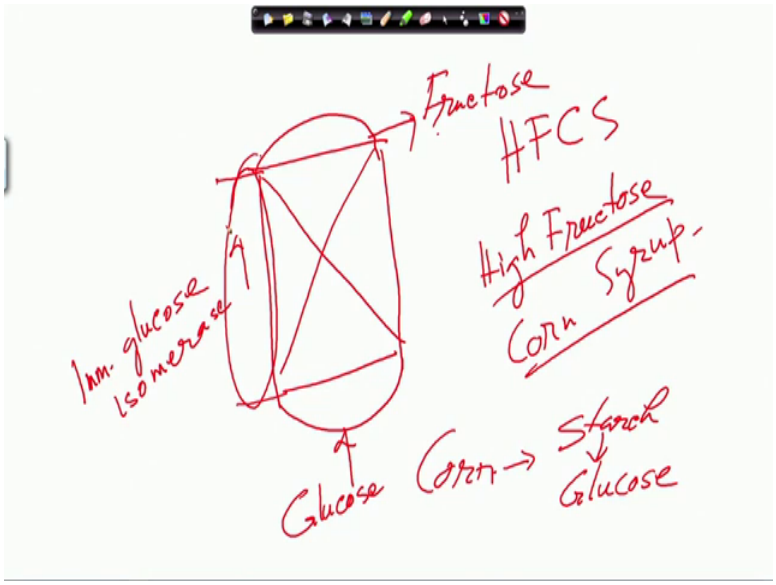

Because I told you that that you know that that one of the one of the major application of the immobilized enzyme system in the western country is the High Fructose Corn Syrup, High Fructose Corn Syrup production. So, what we what we do here as we know Corn , what what is contain. It contains Starch material am I right Starch. So, when it undergoes the saccharification process, this produces glucose. Now this glucose when it pass through this immobilized column, this is the immobilized column what is content the immobilized glucose isomerase enzyme, glucose isomerase. So, this is this is. So, you

are passing glucose here and here it producing fructose, am I right. This is how it is produce. So, this is use for the production of High Fructose Corn Syrup and High Fructose Corn Syrup largely use in the confectionery industry in the western country; largely it is used to that you know as a sweetening agent in the western country. Our country we do not use much, but the western country it is lot of application that we have. Now this problem; this is related with this problem that you know particular issue.

If you pass this glucose solution in this column and converted to fructose, now question come that what for a desire conversion? What is that should be the height of the column? This is how you can design how you can design the column that, what should be the height? This, this, this. So, defined height we have to we have to find out that, what size is there in the column? This problem is very interesting. So, you try to understand.

So, what is here? That you see that the glucose is converted to fructose by using immobilized glucose isomerase enzyme. This you can correct it this is, s you know that isomerase enzyme. The find out the height of the immobilized enzyme column, following data's are given. The diameter of the column is. So, when you when you say that this column. So, this column the diameter this is this diameter is about 5 centimeter am I right 5 centimeter. The particle size is 30 to 40 mesh. What do you mean by 30 to 40 mesh. Mesh means what? Mesh approximately the 30 mesh means approximately not exactly, approximately this is the pore size should be 1 by 30 inch.

Now 40 mesh means is approximately 1 by pore size; should be 1 by 40 mesh that mean that; that means, what suppose you have this is the sieving plates and you have you have here 30 mesh and here 40 mesh am I right? Now, when you pass your particle here, the it will pass through 30 and written by 40. So, though your particle size lies between; between 30 mesh to 40 mesh, because we pass through 30 and written by 40, so that is the 1 by 40 means smaller size. So, this is how the industrially how the particle size can be express the 3 by 40 mesh. Now this is the approximately equal to 0.71millimeter average diameter which we express as d p. Now, feed rate, now here, you are continuously passing the liquid there is the feed rate, you are passing liquid this is about 500 milliliter per hour am I right and glucose concentration at 6 ratio. So, this is operated at 60 degree centigrade because and the glucose concentration here, this about 50 grams per liter.

So, you can consider 50, 500 gram per litre. Am I right? Now glucose conversion efficiency that mean 60 percent of glucose will be should be converted into the into the fructose here in the product. So, that you can we can easily find out. And what is the viscosity of the liquid? 3.6 centipoise at 60 degree centigrade. And the feed density is 1.23 gram per liter at 60 degree centigrade. As substrate diffusivity D equal to 0.21 into 10 to the power minus 5 centimeter square per second at 60 degree centigrade. And void fraction is 0.35. Now, what do you mean by void fraction? Void fraction means, suppose that you know that this is the particle, am I right?

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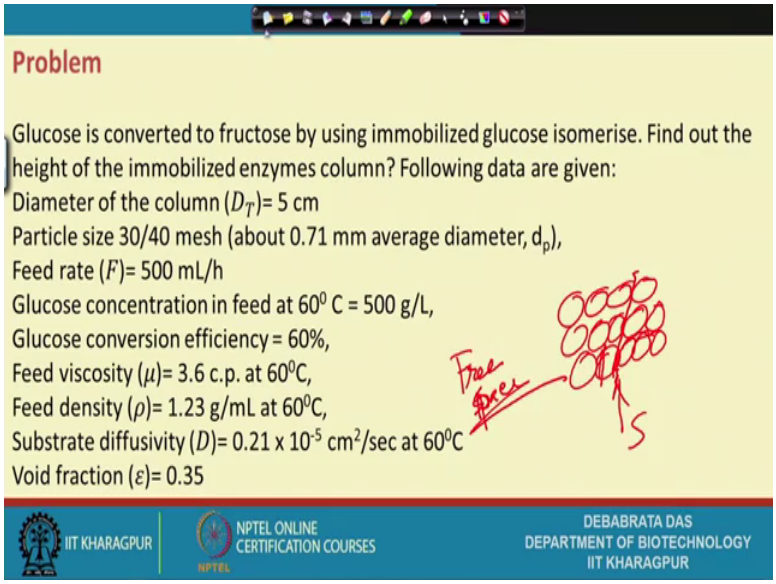

This is the particle that you know we fix in the column, this is particle there lying with each other due to the gravitational force of attraction when, now when you pass here substrate; substrate is the liquid form. It always pass through through the void space. Now cannot penetrate the solid matrix, but whatever the void space is there, it will go like this am I right? So, liquid is moving only through the void space like this. So, that is that is the void fraction. That is 30 percent of the of this solid matrix will be free space. Void fraction mean it is considered as the free space. So, that where where the liquid can travel you know free space where the liquid can travel. So, that is I have a 0.35. So, these are the different parameters are given.

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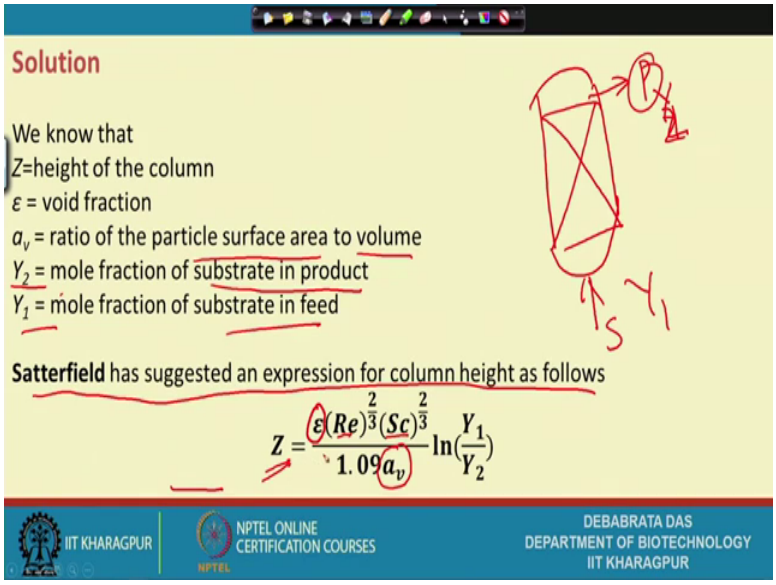

Now, let us see how we can solve this. Now, now what this will be using one equation that is Satterfield has suggested the expression for the column height as follows. The that you know this scientist, he determined that kind of correlation with the flow characteristics of the fluid with the with the that size, the height of the column. And during his research work, he find out this is quite applicable for the determining the height of the immobilized column. Now what he says that is the (Refer Time: 20:20) Z is the height of the column equal to Epsilon; Epsilon is what?

This is the void space and R e is the Reynolds number, this is the S c is the Schmidt number and a b is the that that ratio of particles surface area to volume and Y 1 and Y 2 is called Mole Fraction of Substrate in product; that means, Y 2 is the mole fraction because if you have column like this, so, you are passing your substrate like this product like this. So here, the mole fraction of the substrate is Y 1 and here Y 2 and here Y 1. So, Y this is the input, input is this is a feed this is the Y 1 is the mole fraction of the substrate and mole fraction of the substrate at the product that here it is Y 2 this is what he has. So, this is the correlation that we have. Let us see how we can solve it.

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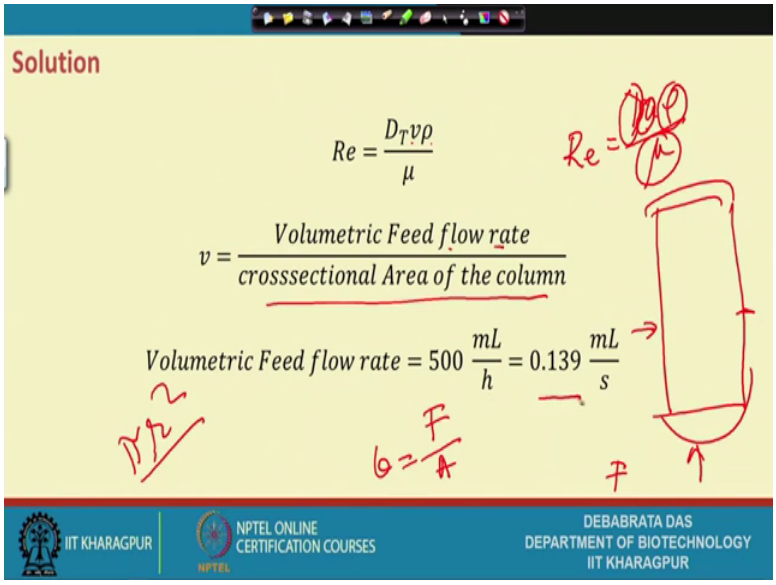

Now, first we shall have to find out the Reynolds number. Reynolds number is the what D u rho by mu; because we know that that is the Reynolds number is R e equal to this is D u rho by mu. This is D is the usually the diameter of the particles and mu u is the velocity of the liquid, rho is the rho is the density of the liquid and mu is the viscosity of the liquid. Now, here the v is the here, d t that v into rho by v, where v is the volumetric feed rate, this the velocity divide the cross-sectional area of the column. So, suppose this is the column and we are passing the liquid with a some flow rate and how we can find out the velocity?

Velocity here velocity is v there saying and v will be equal to what? The flow rate divide by cross sectional area because whatever what is the cross sectional area of the cylindrical column is the pi r square, pi r square is the cross-sectional area. So, we can find out the velocity. So, this is how we can volumetric flow rate is this that ah, but when we do all these calculation one thing we shall have to remember, the unit the we have seen the different units there and there that unit the same units we shall have to show, that is why the flow rate is given 500 milliliter per hour. This is to be converted into milliliter per second like this.

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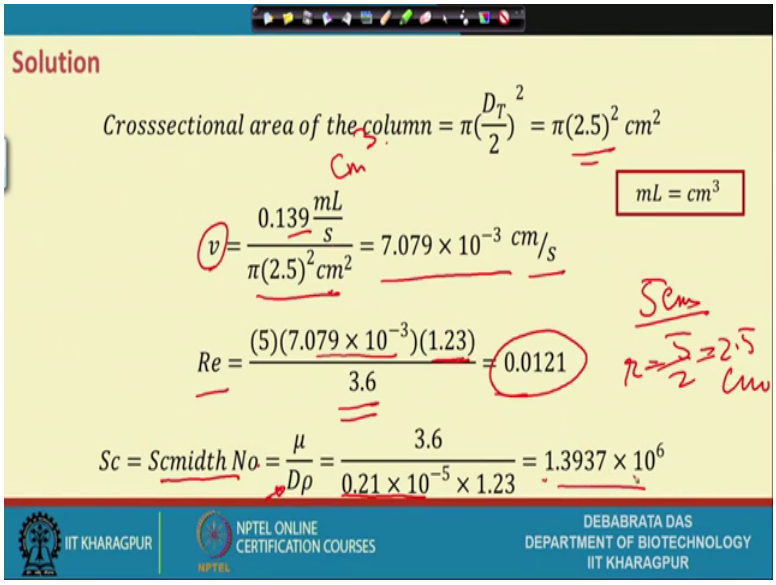

Now, that cross sectional area of the column, how you can calculate? That the cross sectional area diameter is about 2.5 centimeter, am I right? So, not 5 centimeter. Now, if diameter is 5 centimeter, what will be the radius? Radius will be 5 by 2 that is 2.5 centimeter, am I right? So, this is exactly what you has reason. The pi r square I told you that is the cross section and ah. So, what will be the velocity? Velocity is equal to that this is the flow rate 1ah 0.138 milliliter per second and this is the area. Then this will be the centimeter. What is the milliliter? Milliliter means centimeter cubed am I right that. So, this is centimeter square and this is centimeter. So, your square will calculate, it will be centimeter. So, it was centimeter per second. What is the Reynolds number? Reynolds number that we already we have given the D u rho that by mu that D is the diameter of the that vessel that we have. And that u is the is the velocity of the then and this is the density and this is the viscosity. And this value is coming this. And if you if you this Schmidt number that you with mu by D into rho. Now, this D is not the diameter. This is the diffusivity and this diffusivity that has been given in this problem is 0.21 into 10 to the power minus 5. This is the mu is equal to 3.5, this is equal to this and. So, Schmidt number is coming about 1.3937 into 10 to the power 6. So, this Schmidt number you can easily calculate.

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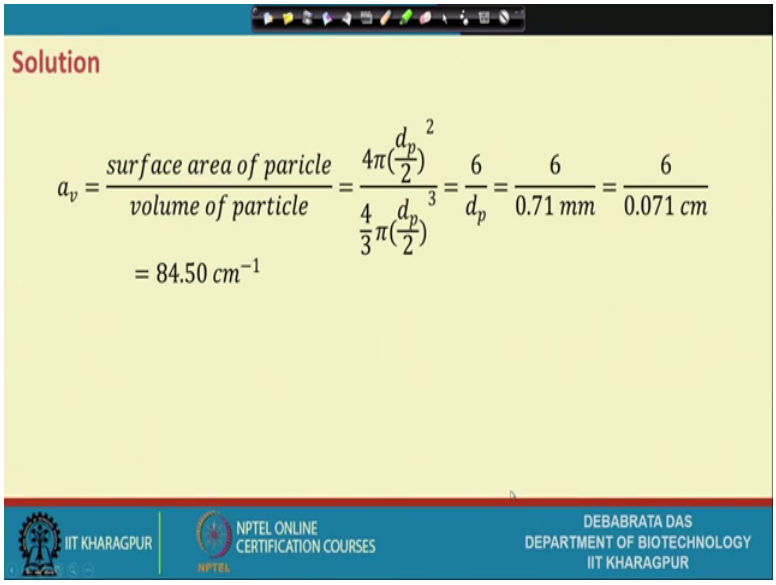

Now, if you if you were then we shall have to find out another parameter, what is the a v? What is a v equal to surface area of the particle divided by volume of the particle? Now, what is the surface area of the particle? This is 4 pi r square am I right? And this is 4 by 3 pi r cube this volume of the, so, as the spherical particle. So, this value is coming around 84.5 centimeter; per centimeter.

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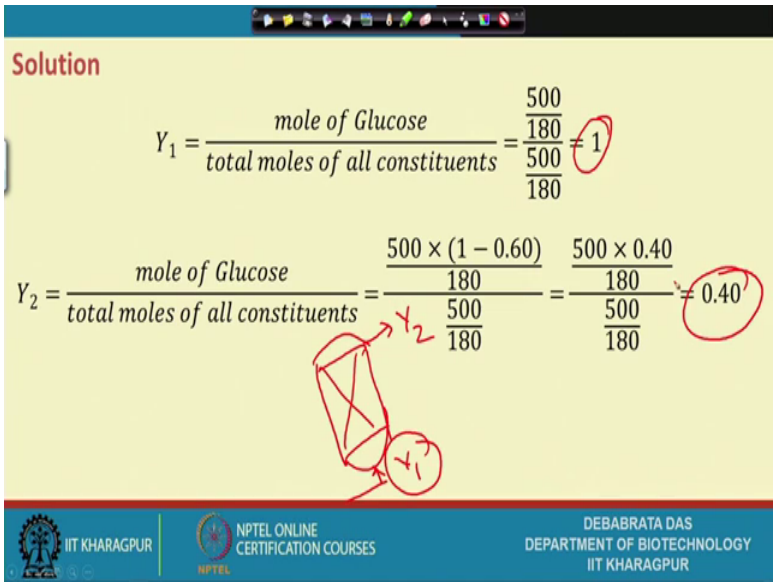

Now, and mole fraction, this is how you can calculate mole fraction in case of that you know that that this is the column and here this is the input liquid am I right? This is the Y 1 and this is output is Y 2. The mole fraction of substrate because, we consider all substrate at present there in the form of glucose, so, this should be 1. And in the outgoing

section 60 percent is converted that mean how much will remain? That is 40. So, mole fraction will be of the substrate, will be 0.4.

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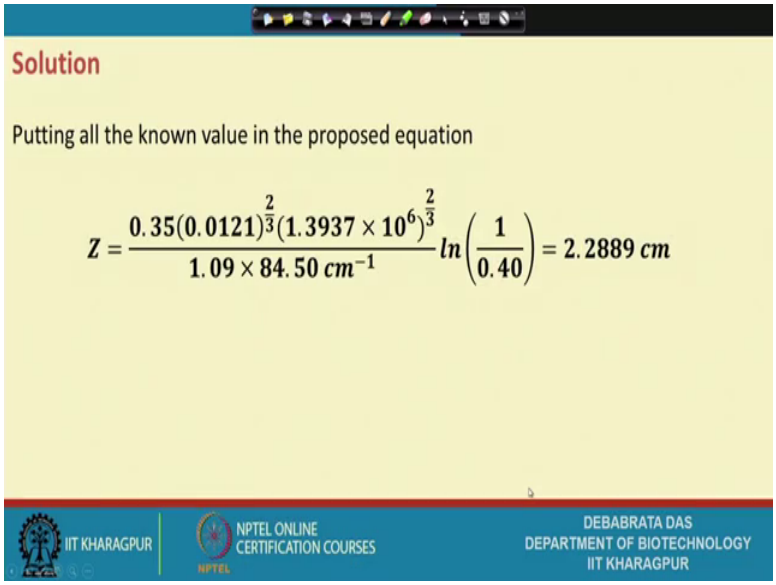

So, now if we have all the all the data's we have and we put the value in this equation then will find, the height of the column will come around 2.2889 centimeter. So, this is the how we can we can find out the height of the column. We can easily of immobilized system we can easily find out the so, ah. So, in this particular lecture, we try to cover some kind of numerical problem; because we first we find out that when you use some kind of cells and that cells undergo respiration that how much oxygen uptake by the cells is there that diffusion problem.

And we try to find out whether it is diffusion control. And another, we try to find out that in case of enzymatic reaction whether that is that is diffusion control that we try to find out and whether it is diffusion control and reaction rate control and finally, we try to find out that industrially when you apply this immobilized enzyme system, we put the enzyme in a column and continuously we produce product. So, question comes for getting a desire amount of substrate conversion how we can determine the height of the column? So, I try to explain one problem related to that, I hope you understand that how it related to the flow characteristics of the fluid, we use the Reynolds number, Schmidt number with this try to evaluate the volume of the of the immobilized column.

Thank you very much.