Course on Industrial Biotechnology By Prof. Debabrata Das Department of Biotechnology Indian Institute of Technology Kharagpur Lecture 13 Enzymatic Reaction Kinetics (Contd.)

Let me continue this enzymatic reaction kinetics

(Refer Slide Time: 0:23)



And now I am planning to discuss some problems on enzymatic reaction kinetics now first problem that we are going to discuss that is an enzyme which came 1 into 10 to the power minus 3 M was assayed using an initial substrate concentration of 3 into 10 to the power 5 M after 2 minutes 5 percent of the substrate was converted. How much substrate will be

converted after 10,30 and 60 minutes in the last presentation you can remember I I derive a equation equation is this that is.



(Refer Slide Time: 1:12)

Vmax into T equal to S zero minus S km Ln S zero by S so you know this equation this is the correlation between time and the substrate concentration now here this point that you know first case they are saying that after 2 minutes 5 percent substrate converted to so what will be the value of S? S will be equal to point 95 into S zero am I right? So if you put this value then you can easily determine the value of a max Vmax.

Because you know everything is known S zero value , know Km, know S, you know T, you know you can easily calculate the value of Vmax and once you know the Vmax then you you

put the value here then you have only this ID have to unknown T and S now in the second case that are saying.

(Refer Slide Time: 2:17)



how much substrate will be converted in the 10 minutes 30 minutes and 60 minutes.

(Refer Slide Time: 2:35)

 $U_{map} = (s_0 - s) + k_m l_m \frac{s_0}{s}$ $2 min, s y. sul., s = 0.95 \times s_0$ $U_{map} = ?$

 $U_{may} = (s_0 - s_) + k_m k_m \frac{s_0}{5}$. $Z_{min}, s_1, s_2, ..., s_2 = 0.95 \times s_0 \quad X = \frac{s_0 - s}{s_0}$ $U_{may} = ?$

So what I can do I can I can I can I can assume the fraction of substrate converted because and then I can the fraction X equal to what is 0 minus S by S zero. So I can assume that that you know fraction of substrate maybe 20%, 30%, 40%, 50% and if we assume that then we can we can we can find out the value of S here and find out at the at what circumstances your left hand side will be equal to right hand side. So this a trial error problem this is low on the basis of trial error we can solve this problem let's us see how you will solve it here.

(Refer Slide Time: 3:14)

Given, 5% conv Therefore, S= 0 S _o = 3 Putting these v	version in 2 min. 0.95 [°] S _o x 10 ⁻⁵ M alues in Eq. 1, we get v _{max} = 2.64 x 10 ⁻⁵ M/min			
<u>Case-l</u> : For t = 1 By trial and err then in the Eq. L.H.S. = R.H.S. Therefore, com	L0 min or method, it has been found that when x = 0.77, 1 version will be 23%			
Case II & III Similarly, % conversion after the time 30 and 60 mins are 54% and 80% respectively.				
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The initially we were considered 5% conversion in 2 minutes is equal point 5 into S zero so S zero equal to this S zero is this much. So here V Max is coming this. For now 10 minutes trial error method you have been found if X is point 77 then equation left hand side would be right inside point 77 means 23% conversion take place. That then in case of 30 and 60 minutes is

this 54 and 80% respectively so this is this is we this problem can be solved very easily because if you know this equation and if I assume this is the X-ray diffraction.

(Refer Slide Time: 4:01)

 $U_{may} = (s_0 - s_0) + k_m l_m \frac{s_0}{s}$ = 2 min, sy. sul., $s = 0.95 \times s_0$ $U_{may} = ?$

So is the X value X is the fraction of X that is converted with we can assume 10%, 20%, 30%, 40% and we can find out and under what circumstances left hand side is equal to right hand side. Second problem that we have.

(Refer Slide Time: 4:15)



Enzyme E catalyzes the fermentation of substrate A the reactant to product R okay. The first the find the size of mixed flow reactor needed for 95% conversion of reactant in a feed stream 25 litre per minute of reactant 4 moles per litre and enzyme the Kinetics of the of the

fermentation of this enzyme are given by this. This is so this you know that Vmax value and this is the KM not KM value they find out this correlation is like this so we can we directly use this equation find out that how what should be the size of the let us see how we can find it out.



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Now what is what is tou CSTER we have already we have already find out tou CSTR.

(Refer Slide Time: 5:20)



 $U_{map} = (s_0 - s_0) + k_m k_m \frac{s_0}{s_0}$ $Z_{min}, s_1, s_2, s_3, s_2 = 0.95 \times s_0$ $X = \frac{s_0 - s_0}{s_0}$ $U_{map} = ?$ $J_{CSTR} = \frac{S_0 - S}{(-m_R)}$

Tou CSTR is equal to S zero minus S by minus RS am I right? Then minus RS is a minus RA whatever you there saying and this is maybe the CA zero by CA by like this so if you consider then here you you have if you look at this.

(Refer Slide Time: 5:47)

2. Enzyme E catalyses the fermentation of substrate A (the reactant) to product R. Find the size of mixed flow reactor needed for 95 % conversion of reactant in a feed stream (25 L/min) of reactant (2 mol/L) and enzyme. The kinetics of the fermentation of this enzyme concentration are given by					
- <i>r</i> _A	$=\frac{0.1C_A}{1+0.5C_A}\frac{mol}{Liter.min}$				
	A R				
	i courses				

95% conversion, 95% conversion means what what will be the S value S value will be point 05 into S zero, am I right? 0.05 into S zero so we have we have initial substrate reactant is 2 moles per litre.

(Refer Slide Time: 6:08)

$$\begin{aligned} \mathcal{G}_{may} \stackrel{p}{\leftarrow} &= (s_0 - s_0) + k_m f_m \frac{s_0}{s_0}, & x = \frac{s_0 - s_0}{s_0}, & x = \frac{s_$$

The initial substrate concentration so we can get this S value then we can we can put these here in this equation and we can find this is 199. 5 minutes that is the Tou CSTR and tou CSTR equal to V by F and what is the flow rate they have given this if they have given this is 25 litre per minute so I so you can easily calculate the you can easily calculate the volume of the reactor you can easily calculate in this in this you can always very easily determine that what is the volume of the reactor this is how we can solve this.

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(Refer Slide Time: 6:58)



This is a we are we are we calculate find out it is 4987.5 litres so you can easily calculate the volume of the reactor and this is very much required for any kind of reaction because industrially suppose we want to produce any kind of product and went a walk any kind of enzyme how we can determine the size of the reactor that we can easily estimated like this so you know this is the.

(Refer Slide Time: 7:24)



This is one of the and next problem is very it directly associated with the industrial problem and this problem is like this the glucose in converted into fructose by using glucose isomeric enzyme and while the initial glucose concentration is 200 grams per litre and degree of conversion is 60% calculate the volume of volume needed volume of the reactant needed to produce 10 kg of fructose per day by glucose isomerise in a batch reactor CSTR and plug flow reactor so this this like this.



(Refer Slide Time: 8:09)

So here what is the substrate we are using substrate we are using this is the glucose am I right? This is the glucose and what is the product we are getting here product were fructose now how much product we are looking for the basis10 kg fructose per day this we want to produce so so should have to we for producing 10 kg of fructose per day by using same enzyme how what should the volume of the batch reactor.

What should the volume of the CSTR what should be this he volume of the plug flow reactor this is the problem that we have the first let us assume this is a batch reactor batch process if you the batch process now other values are there that Michaels menten constant.

That reaction that Vmax is 1.5 into 10 to into 10 to the minus 2 kg per second per meter cube.

And KM is 5 kg per metre cube why P by S means that is the stichometry of the process that is that the one kg of fructose produce form 1 kg of substrate glucose down time they assume to be for the batch process assume to be 4 hours activity of the biocatalyst can be ashamed to be constant in time this is the assumption that have we have given now.

(Refer Slide Time: 10:01)

Given data , initial substrate concentration, $S_0 = 200 \ {}^g/{}_L = 200 \ {}^{kg}/{}_{m^3}$
degree of conversion, $X_S = 0.60$
$v_{max} = 1.5 \times 10^{-2} kg s^{-1} m^{-3}$
$K_m = 5 \ kg \ m^{-3}$
$Y_{P/s} = 1 \ kg \ kg^{-1}$
downtime, $t_d = 4h$
i. Batch reactor
It is known that, $t_{batch} = -\int_{S_0}^{S} \frac{dS}{(-r_S)}$
as it follows M-M kinetics $(-r_s) = \frac{v_{max}s}{\kappa_m + s}$
Integration yields
$t_{batch} = \frac{1}{v_{max}} \left[-K_m \ln(1 - X_S) + X_S S_0 \right]$
Putting all the known values
$t_{batch} = \frac{1}{4.5 \times 40^{-2}} \left[-5 \ln(1 - 0.60) + 0.60 \times 200 \right] = 8305.43$
1.5×10^{-2} = 2.307 h
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Now this is a first we should have to write all the initial data it has been S zero os like this now S zero is very interesting.

(Refer Slide Time: 10:11)

Substants : Glucose Product : Franctoso . Basis : 10 kg functoso/d . Batch 5. Substructs : Glucose Product : Franctoso . Basis: 10 kg functose/d. 50 = 200 2 × 1 × 1kg $= \frac{200}{m^3} \frac{k_{\rm p}}{m^3}$

That S zero because this is all all SI units so it is 200 gram per litre 200 gram per litre now if I want to convert it to the (())(10:23) how we can do that? This is equal to 1 kg equal to how much 1000 gram no it is I want to convert 1 kg equal to 1000 gram and I want to convert it to the cubic metre one cubic metre is equal to how much litre 1000 litres 1000 litres so this is like this.

So this this will cancel the little little little little will cancel Gram Gram will cancel the unit will be what? 200 kg per cubic metre to gram per litre I can be converted to the KG per this is SI unit I can write because all units in the SI unit. So this you have to initially take into

account when you do the the calculations now in the batch process what you know that T batch how we can write this is equal to minus DS by minus RS so this has been given here.

(Refer Slide Time: 11:47)

Given data, initial substrate concentration, $S_0 = 200 \ g/L = 200 \ kg/m^3$ degree of conversion, $X_S = 0.60$ $v_{max} = 1.5 \times 10^{-2} \, kg \, s^{-1} \, m^{-3}$ $K_m = 5 \ kg \ m^{-3}$ $Y_{P/s} = 1 \ kg \ kg^{-1}$ downtime, $t_d = 4h$ i. Batch reactor It is known that, $t_{batch} = -\int_{S_0}^{S} \frac{dS}{(-r_s)}$ as it follows M-M kinetics $(-r_s) = \frac{v_{max}s}{v_s}$ Integration yields $t_{batch} = \frac{1}{v_{max}} \left[-K_m \ln(1 - X_S) + X_S S_0 \right]$ Putting all the known values 1 $t_{batch} = \frac{1}{1.5 \times 10^{-2}} \left[-5 \ln(1 - 0.60) + 0.60 \times 200 \right] = 8305.43 s$ = 2.307 hNPTEL ONLINE CERTIFICATION COURSES

So minus RS equal to what Vmax S by KM plus S now we have we we we can easily we we have we have already derive the equation for T batch 1 by Vmax minus KM this 1 minus XS XS XS SSS is a fraction of substrate that is converted and we assume that 60 percent conversion of substrate sot we have initial substrate concentration also we have given the we can easily find out that for the 60% substrate conversion how much time is required with this is 2.307 hours we can we can easily calculate and find out 2.307 hours now once you find it out.

(Refer Slide Time: 12:46)



Then then we can find out the T total time T total time will be what T to 2.307 plus 4 that is 6.307 now this will be hours now I told you in the that number of positive power back because when we when we do any calculation for the back system first we should have to find out how many how many batch you want to operate per day because you know calculation should be on the basis of per batch because per batch is this 24 hours.

If you divide by total time then you can easily find out the number of batch you can operate per day so then you can find out how much product you have to produce per batch once you know the amount of product formation per batch then you can easily find out the volume of the reactor because how you can find out here it is very simple.

(Refer Slide Time: 13:49)

$t_{total} = t_{batch} + t_{d0wn time}$ $t_{total} = 2.307 + 4 = 6.307h$ Now, no of batch per day= $\frac{24}{6.307} = 3.80$ Base: 10 kg of fructose (P) per day Product to be produced per batch= $\frac{10}{3.80} = 2.63 kg$ As, $Y_{P/s} = 1 kg kg^{-1}$, substrate required per batch= $2.63 kg$ For $X_S = 0.60$, actual substrate required per batch= $\frac{2.63}{9.60} = 4.38 kg$				
Volume of reactor required = $\frac{actual substrate required}{initial substrate concentration} = \frac{4.38 kg}{200 \frac{kg}{2}} = 0.022 m^3$				
ii. CSTR $\tau_{CSTR} = \frac{S_0 - S}{(-r_S)} = \frac{(S_0 - S)(K_m + S)}{(v_{max}S)} = \frac{S_0 X_S \{K_m + S_0 (1 - X_S)\}}{v_{max} S_0 (1 - X_S)}$				
$\tau_{CSTR} = \frac{0.60 \times \{5 + 200(1 - 0.60)\}}{1.5 \times 10^{-2} \times (1 - 0.60)}$ $\tau_{CSTR} = 8500 s = 2.36 h$				

Suppose 10 kg of fructose per day this is produced and total time total number of batch is how much 3.8 now if you divide by 3.8 then per batch how much product we are supposed to produce their 2.63 kg know that means how much substrate is required is 2.63 kg because one gram Fructose one gram fructose produced from 1 gram of glucose so amount of glucose requirement will be same 2.363 Kg.

Now what is the percentage conversion 60% the substrate required for batch will be how much this is divided by point 6 that is 4.38 kg now here though I what I told you that volume of the reactor how we can calculate the actual required required per batch this is 4.38 kg divided by the initial substrate concentration that is 200 kg per cubic metre so it is coming around point 02 to cubic metre.

Now if you multiplied by thousand you will get the 22 litres so in case of to get 10 kg of fructose per day in a particular in a batch process you required the volume of the batch reactor should be 22 liters that will that is that is the solution of this. Now later see how we can find out the volume of the CSTR volume of CSTR also we can similarly calculate it is very simple.

(Refer Slide Time: 15:35)



Because it tou CSTR equal to S zero minus S minus RS am I right so minus RS value we already know this is a Michaels menten equation this is here that you can see that VMax as KM Plus S this is given here now if you're then you are you put the conversion factor here then we we can we can change that equation like this you can easily change this equation like this and and then then we can put the values of the different parameters that we can easily calculate let me show you how we can derive this equations.

(Refer Slide Time: 16:24)



This is equal to S zero minus S this is VMax has KM plus S we write the this is equal to S zero minus S Km plus S plus Vmax S now what are you what is X equal to what S zero minus S by S zero That is equal to 1 minus S by S zero. S by S zero equal to 1 minus X I can write like this now here what we can do we can we can we have we know the substrate concentration am I right?

Because the initial service 60% of the substrate is converted to product so we can easily find out that substrate concentration because in the CSTR under steady state condition substrate concentration we can put the value directly and then you can solve you'll get the same figure even you don't have to do this kind of thing you have to get the same figure even. Because your substrate substrate concentration initial substrate concentration was how much? 200 kg per cubic metre so your 60% conversion he is there to so how much substrate will be remaining this is 200 into this is 1 minus 16 that is equal to 200 into point 4 that is 80 kg per cubic metre this is S value S value will be like this S value so if you put this S value here S zero.

And km and Vmax value is known we can also find out the value of this and once we find out this this is equal to V by F and what is the F how we can calculate F.

(Refer Slide Time: 18:59)

 $t_{total} = t_{batch} + t_{d0wn time}$ $t_{total} = 2.307 + 4 = 6.307h$ Now, no of batch per day= $\frac{24}{6.307}$ = 3.80 Base: 10 kg of fructose (P) per day Product to be produced per batch = $\frac{10}{3.80}$ = 2.63 kg As, $Y_{P/s} = 1 \ kg \ kg^{-1}$, substrate required per batch=2.63 kg For $X_S = 0.60$, actual substrate required per batch $= \frac{2.63}{0.60} = 4.38 \ kg$ Volume of reactor required = $\frac{actual \ substrate \ required}{initial \ substrate \ concentration} = \frac{4.38 \ kg}{200 \ \frac{kg}{m^3}} = 0.022 \ m^3$ = 22 Lii. CSTR $\pi_{CSTR} = \frac{S_0 - S}{(-r_S)} = \frac{(S_0 - S)(K_m + S)}{(v_{max}S)} = \frac{S_0 X_S \{K_m + S_0(1 - X_S)\}}{v_{max} S_0(1 - X_S)}$ $\tau_{CSTR} = \frac{0.60 \times \{5 + 200(1 - 0.60)\}}{1.5 \times 10^{-2} \times (1 - 0.60)}$ $\tau_{CSTR} = 8500 \, s = 2.36 \, h$ NPTEL ONLINE CERTIFICATION COURSES IIT KHARAGPUR

iii. Plug flow reactor (PFR) It is known that, $\tau_{PFR} = -\int_{S_0}^{S} \frac{dS}{(-r_S)}$ as it follows M-M kinetics $(-r_S) = \frac{v_{max}S}{k_m + S}$ Integration yields $\tau_{PFR} = \frac{1}{v_{max}} [-K_m \ln(1 - X_S) + X_S S_0]$ Putting all the known values $\tau_{PFR} = \frac{1}{1.5 \times 10^{-2}} [-5 \ln(1 - 0.60) + 0.60 \times 200] = 8305.43 s$ = 2.307 hBase: 10 kg of fructose (P) per day For $Y_{P/S} = 1$, substrate required per day= 10 $\frac{kg}{d}$ As $X_S = 0.60$, actual substrate required per day= $\frac{10}{0.60} \frac{kg}{d} = 16.67 \frac{kg}{d}$ Actual substrate required per hour= $\frac{16.67 kg}{24 h} = 0.70 \frac{kg}{h}$



I can calculate F how you can calculate F you see that F can be calculated like this actual substrate converted by initial substrate concentration what is the actual substrate required is that you know we have 10 kg 10 kg subsidy required per day 10 kg product per day so actual substrate requirement will be how much 60% conversion is there then the actual substrate requirement is 16.6 KG per day.

Now this is per day so per hour will be this much and this power will be point 7 kg per hour now actual substrate corner point 7 kg per hour and this is initial substrate concentration is 200 kg per cubic metre to flow rate is this this 3.5 into 10 to the power minus 3 cubic metre per hour now if you know if you know this flow rate then you are you are you can easily calculate the volume of the CSTR F into tou CSTR you can multiply I find.

We find out the volume of the reactor is coming around 8.23 litres so in case of you can remember in case of batch reactor we got the volume that 22 litres and in case of CSTR we get the volume of 8.26 litres.

(Refer Slide Time: 20:37)



Now let us see in case of plug flow reactor what happens in the in the case of plug flow reactor your the same expression as you are batch reactor that your tou plug flow reactor equal to DS by minus RS and this from S zero to S so we can we can we can have this minus RS can be substituted by Michaels menten equation and then this equation will be converted to this as a in the batch process.

And this time also similar through the batch process we calculated this is 2.307 hours and 10 kg fructose per day that we want to produce for 10 kg fructose per day how much that you know the product that we have already calculated the before that how much substrate required is point 3 point 7 kg per hour.

(Refer Slide Time: 21:45)



And then flow rate will be same 3.5 into 10 to the power minus 3 cubic metre per hour then we know the same as thay tou CSTR plug flow reactor volume of plug flow reactor by flow rate and then your volume of plug flow reactor is we can calculate at this one.

(Refer Slide Time: 22:08)

Volumetric flow rate $(F) = \frac{actual \ substrate \ required}{initial \ substrate \ concentration} \frac{\frac{kg}{h}}{\frac{kg}{m^3}} = \frac{0.70}{200} \frac{m^3}{h}$ $= 3.5 \times 10^{-3} \frac{m^3}{h}$ Now, we know $\tau_{PFR} = \frac{V_{PFR}}{F}$ $V_{PFR} = F \times \tau_{PFR} = 3.5 \times 10^{-3} \times 2.307 \ m^3 = 8.07 \times 10^{-3} \ m^3 = 8.07 \ L$									
	Batch reactor	CSTR	PFR						
Volume(L)	22	8.26	8.07						
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Now if you compare this 3 reactors you'll find that that that you are batch reactor here volume requirement is 22 litres and CSTR you required for 8.26 litres and plug flow reactor is 8.07 so form design perspective we should go for plug flow reactor because plug flow reactor will require less volume as compared to other two reactor.

So this is how in the Industrial level we can we can we can always calculate always calculate the amount of product that we get from in the in the any kind of processors and how we can determine the volume of the reactor that you can you easily calculate if we have the kinetic information of the enzyme that we have with us but I can give some certain other examples.

(Refer Slide Time: 23:19)

Volumetric flow rate $(F) = \frac{actual substrate required}{initial substrate concentration} \frac{kg/_h}{kg/_{m^3}} = \frac{0.70}{200} \frac{m^3}{h}$ = $3.5 \times 10^{-3} \frac{m^3}{h}$ Now, we know $\tau_{PFR} = \frac{V_{PFR}}{F}$ $V_{PFR} = F \times \tau_{PFR} = 3.5 \times 10^{-3} \times 2.307 m^3 = 8.07 \times 10^{-3} m^3 = 8.07 L$							
	Batch reactor	CSTR	PFR				
Volume(L)	22	8.26	8.07				
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That to make this very clear that I was talking about this this reaction so one interesting feature is that.

(Refer Slide Time: 23:33)





Supposed when the last class in some previous classes I was discussing about the correlation between one by RA versus CA supposed reaction is A to B now if you have we have seen the problem in the (())(23:52) like this now if you have suppose you have initial substrate concentration CS zero and this is the final substrate concentration here CA zero now question comes that what reactor you are going to recommend for this process?

Because if the nature of the correlation between 1 by RA and CA is like this now answer to this question is that we can when you have this kind of correlation we can obviously we can find it is to pattern this pattern is different as compared to this pattern so first we should have to analyse this pattern and then we should go for the analysing that this pattern.

Now we need this when is the look at the this pattern they converse that CA zero to CA dash, CA dash to CA when CA zero converted to CA dash then because I told you when you when you do any kind of design of the reactor our target is to go for the minimum minimum size of the reactor because more the reactor is bigger if the reactor is bigger our investment cost will be more.

So that will increase the product cost that per unit product cost will be more so here when CA zero is converted to CA so that the thing is that is a like this now in case of in case of plug flow reactor suppose we have in this connection let me tell you that always the continuous process are good for the industry most of the industry because the productivity as I shown you in some other classes.

The productivity in the in the continuous reactor is more as compared to that of batch reactor gonna be always there so it is in this because because in India I can give the instance that we have more than 250 distillery in India and most of the distillery that is operated in the Batch Mode and 111 1 main purpose of using the batch reactor is that is very easy to operate.

And one another disadvantage of this particular process is that man power requirement for operating the batch process is very high because why it is very high because I told you that there will be down time you have to take out the material every time you have to clean the vessel you have to refill the vessel so you know that that your productivity also quite less so in the in the industry this is not the this is always we go for continuous process.

(Refer Slide Time: 26:54)





so continuous process if you operate the in the continuous process we have to process one is called CSTR and another is plug flow reactor, CSTR and plug flow reactor now in case of if you go this area under this curve you will find this is equal to tau plug flow reactor because what is the tau plug flow reactor equation is tau plug flow reactor equal to CA zero to CA dash minus DCA minus RA am I right?

So this is like this now at the same time when you do the tou CSTR what is this this is equal to CA zero minus CA dash minus RA so here if you do that then in case of CSTR this area will be tau CSTR obviously in case of this region this is our choice goes to tau CSTR because why CSTR because area of CSTR much less as compared to area of plug flow reactor the so because this is equal to what equal to V by F so is constant for both.

The plug flow and for a particular process but there are since it is proportional to the volume this is the volume is less in the CSTR you are time is less in the CSTR volume will be less but this situation will be different reverse in case of when in case of a CA dash to CA because here the area under this curve will be tau CSTR tau plug flow reactor but area under this this will be tau CSTR.

Which will this area much more higher than this area the the here in this region we call it this we can write that tau plug flow reactor is preferable so what is our recommendation what is our final recommendation of this particular process.



(Refer Slide Time: 29:24)



Our final recommendation is that that we should go for first we go CSTR like this where CA zero is converted into CA dash then followed by plug flow reactor and we get CA so this is like this this the continuous mode this is a plug flow reactor and this is the CSTR so we can easily find out this.

If you have a that correlation between 1 minus RA is and your CA is like this then our recommendation is like this from CA is zero to CA dash will be this and CA dash to CA will be this if you do this then that the volume of the reactor will minimise And our that is our product cost will be reduced thank you very much!