Transport Phenomena in Biological Systems
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Module - 1
Lecture - 4
Mass Conservation for a Microscopic System

Welcome back. In the last class, we looked at the application of a material balance equation or the useful form of the mass balance equation, to derive something useful from a macroscopic system. In this class, we will take that forward.

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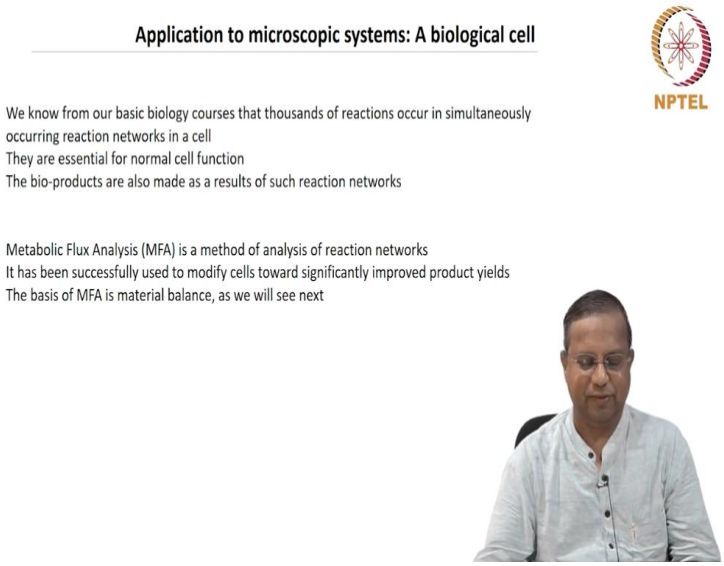

Let me show you the use of the material balance equation, the useful form of the material balance equation to microscopic systems. This is of again direct interest to us. I am going to show you how to apply it to a biological cell. A biological cell, even though it is only a few microns in diameter or its dimension, it still can be considered as a continuum, because of the number of molecules of interest being large enough.

Or, if it is large enough, the rule of thumb is about 100 molecules per cell. If you have something beyond that, you can consider that as a continuum. So, if it is a continuum, then you can apply any of the principles that we are covering in this course. Let us move forward. So, this is application to microscopic systems, a biological cell. You have done a lot of basic biology courses.

And you know that, thousands of reactions occur in the cell simultaneously. In fact, it is, those reactions and those reacting networks, reaction networks, that provide functionality to the cell, to the biological cell. They are also essential for the normal cell function, functionality. The bio products are made as a result of such reaction networks. That is what bioprocess engineers are interested in.

Metabolic flux analysis was a method of analysis of those reacting systems. That was developed, let us say about 40 - 45 years ago; and applied about 30 years ago; to improve the productivity of a biological system, of a bioreactor. Since then, it has been successfully used to even modify cells, genetically modify cells through insights into metabolic changes, towards significantly improved product yields.

The basis for this is metabolic flux analysis. This is a method of an analyzing or one of the methods of analyzing the biological reaction networks. It is nothing but material balance. That is what we are going to see next.

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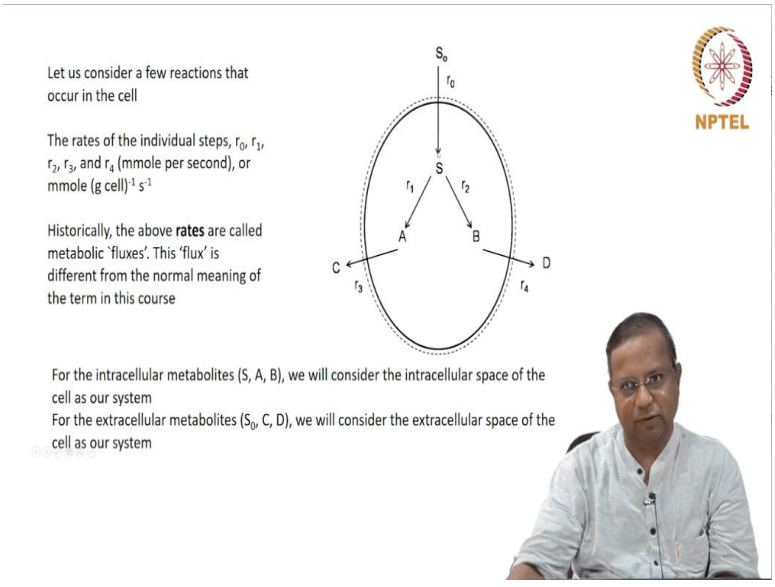

To do that, consider this as a biological cell. I have drawn it nicely as an oval here. You know that the cell can be of any shape and size and so on. Let us consider this to be a cell. And we are going to consider the cell as our system, as indicated by these dashed lines. This is the system boundary here. We are going to consider a typical network, reaction network that is occurring in the cell.

A very, very simplified form of the network. Here, let us say that a substance S_0, a substrate S_0 in the extracellular space, gets taken in by the cell and probably gets converted in the process; to something called S. Yeah, let us say that it gets converted to something called S. The S gets converted to A, through one set of reactions, and to B, through another set of reactions.

And A gets converted to C, which is actually coming out of the cell, an extracellular product. And B gets converted to D through a set of reactions. And D is also coming out of the cell. The rates for these steps; the rates are in millimole per second(mmol s^{-1}), okay, or millimole per gram cell per second(mmol $gcell^{-1}$ s^{-1}). This is more the practical unit, because this is how it is measured. It could be millimole per second (mmol s^{-1}) for principle; it could be mmol $gcell^{-1}$ s^{-1} for practical reasons.

Let us say that the rate of this process S_0 to S is r_0. The rate of S to A is r_1. The rate of S to B is r_2. And the rate at which A gets converted to C and gets pushed out is r_3. The rate at which D gets out of the cell from B is r_4. These are, these, although these are rates, they have been called fluxes or metabolic fluxes, from a historical perspective. That is the term that it began with.

And that is the term that has stuck. Note the unit here is millimole per second (mmol s^{-1}) We will call this flux as an exception in this course, in this context; and maybe in probably couple of other contexts, which we will get to later in the course. The normal meaning of flux in this course is different. That, we will get to soon in the next chapter. For now, let us call this rate as flux.

Fully realizing that we are going to change this definition of flux very soon, to fit into the main theme of the course or the main definitions in the course. For looking at the intracellular metabolites, which are the intracellular metabolites here? S, A and B. For the intracellular metabolites, S, A and B, we are going to consider the intracellular space as our system.

That is what we are going to focus on. The rest, we are going to consider as surrounding. Whereas, for the extracellular metabolites, which are S_0, C and D, we are going to consider the extracellular space as our system. We are going to focus on the extracellular space and do our balances based on that. So, if you consider the extracellular space as our system, the intracellular space becomes our surrounding.

This is not so difficult to understand. It will become clear very soon. But, just keep this in mind. This is a trick that we need to do, to get a consistent set of equations that we can work with; or to get a consistent set of relationships that we can work with. That is the reason why we are doing this.

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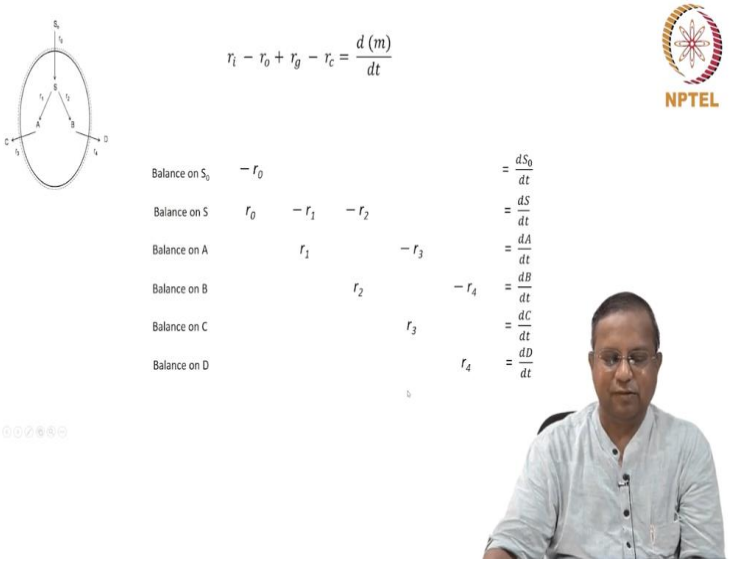

So, this is the cell. The same cell S_0 to S; S to A & B; and then A to C; and B to D here. Now, we are going to apply this equation. This is the useful form of the material balance. Input rate - output rate + generation rate - consumption rate = the accumulation rate of that particular species that we are interested in. This equation we said is very general. We can close our eyes and apply it over a system.

$r_i - r_o + r_g - r_c$ = Rate of accumulation (mass balance equation)

It should work fine. So, that is essentially what we are going to do now. We are going to close our eyes and apply this; and see what the various terms are. So, let us consider the intracellular metabolites first. For the intracellular metabolites, as we mentioned, the cell is our system. And let us consider them one by one. Let us start with S. So, if you do a balance on S, which is essentially writing the various terms in this equation, for the intracellular system.

There is a certain input rate of S. That is r_0.There is no output rate of S. No S is going out. There is no generation of S. We will come to this in a little bit. There is of course consumption of S through the reactions leading to A and through the reactions leading to B. The rates of those are r_1 and r_2 respectively.

$r_0 - (r_1 + r_2) = \frac{dS}{dt}$

Now, let us look at this particular aspect alone. The balance is on S. We said r_0 is the input rate. Here, what has happened is, the process of S_0 in the extracellular space to S in the intracellular space, is a number of individual reactions. All that has been combined into this. And that is normally done in this analysis. A set of reactions are combined into one step, one representative step.

And that is what has been done here. You could look at this as S getting input into this cell at a rate of r_0. Or, S getting generated in the cell from whatever, S_0 getting in and so on so forth, at a rate of r_0. It could be either of those. It cannot be both. If you do it as both, then you are double counting the same thing. So, essentially, we need to avoid double counting.

Therefore, you could either consider such things as either an input or as a generation, because of some complexity involved, you know, S_0 going to S. If it is just A inside, going to A outside, it is a clear output rate. Or A outside, coming to A inside, it is a clear input rate. That is not the case here. Here, because of the way the analysis is done, a lot of things are combined into one step.

And therefore, we need to be a little careful, do not double count. That is the bottom line here. So, let me go through this again. A balance on S; input rate of r_0; there is no output; there is no generation in this case, we have already accounted for in the input. A consumption is through rates r_1 and r_2. And since the r_c is negative, we have $-r_1 - r_2$.

$r_0 - r_1 - r_2 = \frac{dS}{dt}$

Okay, let us move forward. Let us write the balance on A. Is there an input rate of A? No. There is no crossing of A, across the system boundary. Is there a generation rate? Yes. No, there is no, actually, there is no output. At the A, when it goes out, it is actually C through a set of reactions. So, there is no output. There is certainly a generation from S.

There is a consumption here. And therefore, the various terms are r_1, which is the generation rate from S. And r_3, which is the consumption rate of A through this set of reactions.

$r_1 - r_3 = \frac{dA}{dt}$

Balance on B, which is intracellular. Therefore, we are considering this as our system. There is no input rate. There is no output rate. There is a certain generation rate r_2. There is a consumption rate r_4.

$r_2 - r_4 = \frac{dB}{dt}$

We have looked at S, A and B, which are the intracellular metabolites. Now, we have S_0, C and D, which are the extracellular metabolites. When, we said that, when we look at extracellular metabolites, we need to consider the extracellular space as our system. So, that is going to be our focus. So, whatever gets out of the extracellular space, into the intracellular space, will be an output from the system. Whatever is getting into the extracellular space, will be an input and so on so forth.

A balance on C, which is this, would therefore be; you know, there is an input rate. There is no output rate of C. C is not going out anywhere. There is no generation of C. We have already accounted for it in the input. There is no consumption of C. And therefore, r_3, which is the input rate, therefore the mass balance on C can be written as $r_3 = \frac{dC}{dt}$.

I am just aligning the equations like these for a certain reason, which will become clear in the next slide. The balance on D. Why do not you take a second and write it. Then, I will show you what it is. Can you do that? Pause the video if you want now. Write it; and then check it. That might be a little easier for you. Hopefully, you would have gotten this D. We are focusing on with the extracellular space as our system. There is an input rate of r_4 . There is no output rate. D is not going out of the extracellular space. There is no generation of D. There is no consumption of D. And therefore, it is written as $r_4 = \frac{dD}{dt}$.

There is one more, right? There is an S_0 remaining. So, let us do a balance on S_0. I have just placed it here for a certain reason. S_0, if you look at it from the point of view of extracellular space, S_0 is getting out of the system at a rate of r_0 into the extracellular space, which happens to be the surroundings here. There is no input rate. There is an output rate of S_0, from the system to the surrounding. There is no generation rate. There is no consumption rate. And therefore, it is written as $-r_0 = \frac{dS0}{dt}$.

So, there were 6 metabolites. And these are 6 balances that we have written. And these balances, does this remind you of something? You know, I took pains to align things and write this, this way. Does this remind you of something?

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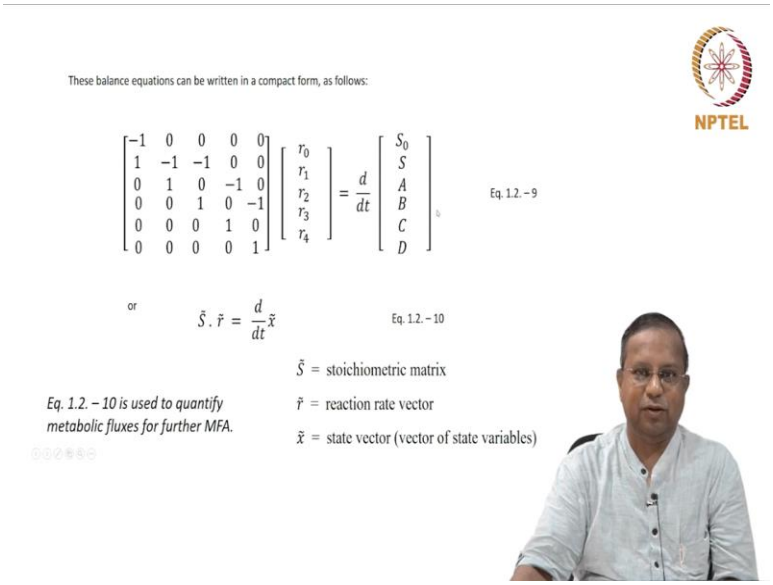

It reminds you of a mathematical way of writing it. They can be written in a compact form as this, as matrices, right? You know how to do matrix multiplication. Consider the first row of the stoichiometric matrix and the rate vector matrix and do the multiplication.

$(-1*r_0) + (0*r_1) + (0*r_2) + (0*r_3) + (0*r_4) = \frac{dS0}{dt}$.

Okay, that was the equation here for S_0. So that we got nicely by this matrix multiplication here, by representing the involved aspects as matrices. We can do that. Okay. So, in one compact way, we have represented the entire set of 6 reactions. In a normal cell and analysis, there could be thousands of reactions; 1000, 3000, 4000 reactions are very common when you do this kind of an analysis. And it is not possible to individually write down and keep track. Even writing down those reactions would take so many pages and it is not going to be very effective.

So, if you write this in a mathematical form, you can just put this into a program. A computer will spit out the results. And you have it. You have whatever you are looking for there. So, that is the reason for converting this into a matrix form. So, I am calling this equation 1.2-9. This is to be consistent with the equation numbering in the book. It will not be continuous in these lectures, as I mentioned earlier.

But this will correspond to the equation number in the book; so, it is easier for you to follow.

$\tilde{S} \cdot \tilde{r} = \frac{d\tilde{x}}{dt}$ Equation 1.2-10.

where $\tilde{S}$ is called the stoichiometric matrix, $\tilde{r}$ is the reaction rate vector and $\tilde{x}$ is the state vector or the vector of state variables(S_0,S,A,B etc.). In this case, it happens to be concentrations in the extra and intracellular spaces. So, using the basic principle of material balance, we have converted metabolism in the cells, a network of thousands of reactions into a form that can be analyzed for various ends; to improve productivity, to understand what is happening inside and so on and so forth.

That is a very hot area of research or a very important area of research even now. From the viewpoint of diseases and so on so forth, a lot is being done. So, the basis of all that is nothing but material balance. And this is to quantify fluxes further, for metabolic flux analysis. This, I have just given you the representation. I have not given you the analysis here.

You need to analyze this further, in the way that you want, for your particular need to come up with insights. So, that is an example of the application of the very powerful principle material balance to a microscopic system, in this case, a biological cell. See you in the next class. We will continue. We would take this further when we meet next. See you then.