IB01x - 3.2 - Basics of the black box model

Previously I introduced you to the process reaction for 1 mol PDO, which is the cornerstone of process design, and whose stoichiometry depends on ratios of q - rates, which brings us to the question of what determines the q - rates?

q-rates depend on the chemical environment of the organism, which is defined by the concentrations of all nutrient components, such as glucose, ammonium, trace metals, vitamins, oxygen, carbon dioxide, product, but also the temperature, pressure and pH. This means that the q-rate of each compound is dependent on all these factors leading to very complicated kinetic functions for qi. This makes it difficult to manipulate the q-value at will. We can simplify such complicated kinetics for the q-rates by controlling T, pressure, pH, which make q-rates depend on all nutrient and product concentrations. This still leads to very complicated behaviour of q-rates.

The magical trick is to design your nutrient supply in such a way that only one nutrient, for example substrate, is limiting and all other nutrients are in excess. In this situation only cs influences the q-rates, if the pressure, temperature and pH are constant.

So what is the basis of the limiting nutrient concept? Suppose you have a cell, in which substrate at concentration cs enters through a transporter, with rate qs. The substrate molecule binds to the transporter, and then the transporter rotates and the substrate molecule disengages at the inside of the cell membrane. The substrate molecule has passed the cell membrane. As you can see there are two modes in which the transporter can be. It can be an empty transporter without any substrate or it can be loaded with the substrate. The transportation speed, qs, depends on the fraction of loaded transporter. And this fraction depends on the concentration of substrate and a binding constant of substrate to transporter.

Let us now show an example for transport of substrate. The fraction of loaded transporter depends on the substrate concentration as a hyperbolic function which tells us certain things about the transport process. When cs equals the affinity parameter Ks then the transporter is 50% loaded. This means that when the extracellular substrate concentration reaches the value of the affinity parameter half of the transporters will be empty and half will be loaded. When the substrate concentration becomes 20 times the Ks value the fraction loaded transporter becomes 0.95 which is almost 1 meaning that nearly all transporters are occupied in transporting substrate over the cell membrane.

Because the loaded substrate/transporter complex unloads the substrate molecule inside the organism at a constant first order rate constant, the substrate uptake kinetics for qs is similar to the hyperbolic function in substrate concentration cs which we just saw. Therefore qs is very close, 95%, to the maximum uptake rate qs,max when the substrate concentration reaches 20 times the substrate affinity parameter Ks. This uptake rate function can now be defined as qs=qs,max*(Cs/(Ks+Cs)) which is a direct result of the fraction of loaded transporter. We can now define three regimes of substrate concentration. First there is

substrate excess regime when Cs>20 Ks. In excess condition the fraction loaded transporter remains close to 1, independently of the concentration Cs. So when there is a change in the substrate concentration there is no detectable change in the fraction of loaded transporter and thus no change in substrate uptake rate. Secondly there is the substrate limiting regime when Cs <20 Ks. Now the fraction of loaded transporter does change with a change in substrate concentration and thus the uptake rate of the substrate depends on the substrate concentration. Finally there is a third regime in which there is no extracellular substrate present. This is called the starvation regime where the uptake rate equals zero.

This uptake rate for substrate also applies for every required nutrient for the microorganism. Therefore, when you design your nutrient supply such that 1 nutrient, e.q. substrate, is in the concentration regime below 20 Ks, and for all other nutrients the concentrations are always above 20 Ks, then the q-rates depend only on cs, provided you keep T, pH and pressure constant.

We come now to last general property of microorganisms which is flux-coupling due to the pseudo steady state of the cell inside. This is a result of the very short passage time of molecules inside organisms. To convert substrate into carbon product, biomass or CO2 it only takes typically under 1 minute. This short passage time is due to low concentrations, 10-2 to 10-6 mol/L, of metabolites in the pathways inside the organism. A change in cs, the limiting substrate concentration which changes qs, immediately (within 1 minute) changes μ and qp and all other q-rates. The cells inside can be seen as one pseudo steady state box where every change in uptake immediately (< 1 minute) changes the other q-rates going in and out of the cell. A big advantage now is that not only substrate uptake depends on the substrate concentration due to substrate limiting conditions. But also the μ, qp and all other q-rates only depend on the limiting substrate concentration cs. We do not have to learn about complicated pathways inside the cell. This pseudo steady state situation inside cells allows us to set up a black box model where we only look at uptake and secretion rates and do not have to look inside cells.

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