IB01x - 2.5 - Learning about the microorganism: qe rates and chemostat

Welcome back.

So far you have learned how to improve your quantitative knowledge about processes in the fermenter. The key insight is that you must calculate rates using balances and measurements.

I have shown you how to get Rs, Rx and Rp.

But also, RO2,

RCO2 and essentially there are many more. Moreover, you learned, using element conservation principles, to check the presence of unknown mechanisms for possible economic improvement.

In the following two units we are going to shift the focus from the fermenter to the microorganisms which are present in the fermenter. All the previously determined rates are realized by a certain amount of microorganisms present in the fermenter. In order to judge the properties of the organism, for example:

Is it a high- or low-producing organism?

We need to standardize all production and consumption rates for the amount of microorganisms present in the fermenter. These rates are what we call the biomass specific rates, the q-rates. The quality of an organism is reflected by these q-rates.

In case of our example, we can calculate the biomass specific rate q for a compound by dividing the rate R of that compound by the total amount of biomass in the fermenter , which is the broth volume VL times the biomass concentration cx. By doing so we obtain qs, qp, qO2, qCO2 and μ. You might ask you self, why do we have a different symbol for qx? This is history and tradition, so everyone calls it μ, which is also known as the growth rate. In order to get the value for the q-rate of a compound we need to measure the broth volume VL, biomass concentration Cx and everything else to get the rate of that compound from its own compounds balance as discussed extensively in previous units.

Q-rates are important because they inform you about the performance of the organisms. But to underscore the importance of using q-rates, let me give you one warning: In practice people compare, for example, the quality of organisms by looking at product concentration; then they draw their conclusions. This is a very dangerous procedure.

The following example shows my point. Let's calculate the product yield for the following experiment. Which means we are interested in moles of product produced per mole of substrate consumed. This example shows that if you take the concentration approach, by just dividing concentration differences things will go seriously wrong, because the volume flows in and out are different in this particular experiment. However, if you use the correct

rate approach, you will see that the wrong concentration approach leads to an underestimation of the yield of 33%. Which might have serious consequences it if goes unnoticed.

Now another experiment, where we add product in the feed of our fermenter with a concentration of 100 mol / m3. So this increases the product concentration in the fermenter obviously and allows us to see if there is product inhibition. , If you now would only look at the product concentration in the broth outflow, you could conclude: Yes there is inhibition, because the product concentration in the broth outflow only goes up by 83.3 mol/ m3 and not the expected 100 mol/m3, which was added in the inflow. However, this conclusion is also wrong. The only correct approach is to calculate the rates, in this case qp, and from these you can conclude that there is no inhibition at all, because qp doesn't change in experiment 1 and experiment 2.

Now, if you want to compare the performance of microorganisms, then my advice is always: Do not compare based on compound concentrations only. Make your balances, do your experiments do proper measurements as you can see from the balances and calculate your q values for two different organisms in the same situation. and then draw your conclusions. This is the only safe way.

One of the most important q-values is μ, because it largely determines the behaviour of the microorganism. So to study the behaviour of our organism we are interested in forcing the organisms to have different μ values.

How are we going do that? An elegant way is to study the organisms is in a chemostat. The advantage of a chemostat is that we can control the growth rate μ. So reset the growth rate, not the organism. In order to explain this, I have to set up the biomass balance, where μ is present.

Here we have our chemostat, with the microorganism. For now we'll only look at the broth phase, because that is where the organism is present. We have Fin and Cx,in, because we could feed for example microorganisms, and we have Fout with Cx ,out , because we must keep the broth volume constant in a chemostat. Finally we have the broth volume and the biomass concentration in the broth, called cx. We assume ideal mixing , so Cx is the same everywhere.

Let's now set up the biomass balance for a chemostat in mol biomass / h.

Once again: Accumulation is equal to: The biomass that is produced , plus the biomass that goes in minus the biomass what goes out . Note that we have replaced the production term of biomass Rx with the biomass specific growth rate μ multiplied with the biomass amount VLcx. Now we can see from this balance what we need to measure to calculate μ, which we have seen many times before.

But now we can take the balance information one step further. Because we do the experiment in a chemostat, at a certain point you get will get a steady state, were concentrations don't change.

The steady state simplifies the biomass balance. In this case the accumulation term will go to zero , because the broth volume VL and biomass concentration cx are constant in time. The biomass balance gives now an explicit relation for the steady state value μ: μ equals Fout cx,out minus Fincx in divided by VLcx.

I'd like to point out now an important aspect from this equation.

Many researchers make implicit assumption such as that Cx,out is equal to Cx, which you would expect with ideal mixing and is true most of the time. However we have seen situations in the lab were Cx,out is larger or smaller than Cx. Another usual assumption is that Fin = Fout, which is certainly not always true. Because for example you add titrant which means Fout > Fin. So be careful with making your assumptions and always measure Cx, Cx,out, Fin and Fout.

Now suppose that you are lucky, and in your case Cx,out is the same as Cx, following measurements. Now we can also set the biomass concentration in the inflow zero. And now you see that this term will go to zero and that the biomass concentration Cx will divide out from the biomass balance. With these two assumptions we see that the growth rate μ in steady state is Fout divided by VL. This is the secret of the chemostat, because this tells us that, with these assumptions, we can come to the lab in the morning, set our Fout and our volume of broth belonging to the μ-value we desire, and after some time the microorganism is forced to grow at this desired μ-value because of the steady state. The next day we can set another μ- value and so on. So we can expose the organism to a range of different μ-values and see what happens to the other q-values.

So a chemostat is an enormously flexible instrument to study the q-rate properties of any microorganism.

So we can, for example, compare different organisms at the same growth rate and we will see whether a mutant has a higher production rate or not.

Now this kind of quantitative insight you can only obtain from chemostat studies.

The other q-rates in such experiments, can also be obtained by setting up the balances for the components, so substrate, product balance or anything balance you like.

So to wrap-up: Q-rates are key performance indicators of the organisms. They are obtained from balances and can be manipulated in the chemostat where we can impose a μ-value to the organism. And finally, never judge the performance of an organism based on concentrations, always set up: balances, do yout measurements and calculate the q-rates.

See you in the next time!