IB01x - 2.6 - Learning about the process and organism: Batch

Last unit we discussed the advantages of using a chemostat to study the performance of microorganisms. In practice the chemostat is a complicated piece of equipment, you have to feed, you have an outflow and you have to keep the volume constant. In practice we also use another type of cultivation, which is batch.

Here we have the chemostat and the batch. The batch is essentially the same cultivation vessel but there is no inh or outflow, which significantly simplifies the fermenter operation. All components like biomass, substrate and other nutrients are added at the start of the batch. In chemostat μ, qs and qp can be controlled by us. This basically begs the question, what happens to μ, qs and qp in batch?

To answer this question about rates, of course we must look at the broth balances for biomass, substrate and product in the fermenter. Here I have my batch fermenter but of course no inflow and no outflow. We have a volume VL(t), and of course concentrations cs(t), cx(t), cp(t) are all time dependent.

First I want to you to think about something. A batch has no in- or outflows. So you might think that the broth volume is constant. But, think about pH control which makes that unlikely, or evaporation of water.

Now let's have a look at this graph for cs.We see cs rises, it increases in time.

That's strange, at first glance, because the biomass concentration also increases, which is solid evidence that the organism is consuming the substrate. So you expect the substrate concentration going down. The explanation is that you are thinking in concentrations, which is wrong and the explanation is that the broth volume is going down.

Such volume changes in batch are common. They can occur when you add acid or base, you evaporate water when you blow dry air through the reactor, or maybe your product is volatile. So the real understanding comes now by setting up the balances for batch.

The batch balances for biomass and substrate follow by realizing that in batch there is no transport. Leading to a balance with 2 terms: accumulation and conversion.

This situation has two consequences: There can be no steady-state and concentrations and volumes are time dependent. However the batch balances always contain the combination VL times cs or VL times cx, which we call the amount of substrate and the amount of biomass. The balance tells us that concentrations do not matter, as I always claim, but that the amounts are important, which are products of concentrations multiplied by volume.

When we plot the amount of substrate and biomass, using the measured time varying volume and concentrations as suggested by the balances, the experiment makes sense. The amount of substrate decreases and the amount of biomass increases, which is exactly what you would expect. So once again, concentrations give you wrong information.

So now the question is, how do we obtain μ in batch. Of course we ned to set up the biomass balance using biomass amounts. Then we need to make the assumption that μ is constant in time, otherwise will not be able to solve the equation.

If you think about it you might remember that microorganisms have a characteristic doubling time.

So a constant μ is usually valid in a proper batch. Usually μ is highest in batch, because all nutrients are present in high concentrations. Therefore we call this μ value the μmax.

The resulting biomass balance with a constant μmax is a differential equation with two variables, Nx(t) and time, and it has a constant parameter μmax.

In order to solve this equation we need to integrate.

This is done by separating variables, by bringing Nx(t) to the left and basically dt to the right and then we can integrate both sides with initial conditions Nx(0) for the left side and time zero for the right side and of course we have also Nx(t) at time t.

The results after integration are: either a log-linear relation for linear dependence of the natural logarithm of the biomass amount in time, so it's not a concentration which is exponential, and an exponential relation for the biomass amount in time.

These relations coming from the biomass balance are needed to calculate μ.

When you have a spreadsheet you can make a non-linear fit of Nx(t)/Nx(0) versus time and then you will find μmax from the data fit. Or, you calculate the natural logarithm of Nx(t)/Nx(0) and plot this number versus time to obtain a linear plot of which the slope is according to the previous result equal to μmax.

μ is not the only biomass specific rate we are interested in. Another question is how do you obtain substrate uptake rate qs from batch. And here we have a problem.

Well we first set up of course the substrate balance for the batch, because that is where qs is sitting as you can see. And we see also that qs is constant and equal to qsmax. We can see that the substrate amount Ns(t) is dependent on the biomass amount Nx(t), which is a nasty exponential function of time.

Solving this differential equation is a lot more challenging. However we can use our previous insights in the biomass balance and use a trick to circumvent unpleasant mathematics.

Both the biomass and substrate balance can be combined in a so-called combi-balance. Here the mathematically inconvenient term Nx(t) is eliminated from the two balances. The resulting combi-balance can be integrated because μmax and qs,max are constant. Filling in the initial conditions gives a result which linearly relates consumed substrate to produced biomass.

If we then plot the consumed substrate against produced biomass, we get a linear relation and the slope we find will give us the ratio of $q_{s,max}$ over μ_{max}. Since we already acquired μ_{max} from the previous biomass balance exercise, we can now calculated $q_{s,max}$.

The same combi-balance approach can be used for calculating $q_{p,batch}$ using the combination of product and biomass balance in the same way.

To wrap-up. We have seen that the broth volume in batch is seldom constant. If we look at the broth balances, they tell us that we should use amounts of substrate, biomass and product, and not their concentrations. When we solve the biomass balance for batch we see that biomass growth is exponential in time, but that is the amount of biomass and not the concentrations in time, allowing us to obtain μ_{max}.

Finally, we can calculate $q_{s,max}$ and $q_{p,batch}$ using combi balance approaches.