IB01x - 2.3 - Learning about the process: Broth balances

Up till now we have discussed why we should use microorganisms as a catalyst and explained which nutrients are needed to cultivate these microorganisms. In this unit we will quantify the production and consumption rates of all nutrients in a fermenter containing microorganisms and see why these rates are important and what they tell us about the process.

Imagine you are working at a production plant that produces PDO. Suddenly your boss comes in your office and is asking for the temperature and pH of the fermenter. You know that these are measured by sensors in the fermenter.

You look at your monitor and you tell him the measured temperature and pH.

The next question your boss asks is the money question:

How much money is earned at this moment, in Euros/h?

Because the most important cost factor is substrate, and income is related to produced product, this question requires that we know how much moles of substrate are consumed per hour and how much moles of PDO are produced per hour. So you need to get these numbers, but how do you determine these rates?

But before we go any further, I would like to show you, in more detail, what a fermenter actually is. It has an aqueous inflow containing the substrate and all the other required nutrients which we discussed earlier. Microorganisms in the broth phase grow on this substrate, but they also convert it into products. The fermentation broth leaves the fermenter through the fermenter outflow. It contains the organisms, the product, by-products made by the organisms and also residual substrate molecules that the microorganisms didn't consume.

If oxygen is required gas can be supplied to the fermenter through the gas inflow and leaves the fermenter through the gas outflow. For optimal growth conditions fermenters are usually mixed, so that the broth has a homogenous composition.

Fermenters can be operated in all kinds of ways, but we will stick to the continuous fermentation for now.

This means that these streams are continuously entering and leaving the reactor.

So how do you calculate the substrate consumption and product production rate?

I've been asking this question to my students for decades now, and the initial answer is always: use sensors, which measure concentrations of substrate and product. And they complement this answer by stating you have to measure the concentration of substrate and product in time. So then I explain my students, when running a continuous fermentation in

steady state plant the concentration of product and substrate are constant in time. But this doesn't mean that there is no production or no consumption.

So the answer to the question:

How do I get my rates?

Is rather difficult, because rates of conversion cannot be measured . You need to calculate these rates using compound balances and the proper measurements. A rate has to be calculated properly, because it provides very important information, it tells you whether your process is economical feasible or not.

Let's make such a balance.

First we need to have a system, this system is defined by boundaries.

Let's define a very simple system, represented by this rectangle In this rectangle certain amount of moles of compound i is dissolved, concentration ci , in the broth, volume VL, of the system. If you multiply them you will get the amount of compound i, Ni in moles.

This Ni remains constant because nothing is added or pulled out.

But now we will add a liquid flow Fin in m3/hour with a concentration of compound i, ci,in . Because there is no outflow, the moles will pile up, it will accumulate, and is represented by this term, dVLci/dt .

This mathematical term represent a change of moles of compound i over time. Now we remove molecules i from the system with, Fi,outcout. a certain amount of moles per hour will now leave the system . The compound balance is:

dVLCi/dt = Finci,in - Foutci,out.

Note that each term of the compound balance has the same unit moli/h.

But now there are microorganisms present in this system, which can consume or produce a certain compound i with a rate Ri.

The compound balance is now:

dVLci/dt = Ri + Finci,in - Foutci,out.

From this balance you see what you need to measure to calculate Ri. You can measure the volume of your broth, VL, in the fermenter, the concentration of the compound, ci, the flow in and flow out and the concentration ci in these flows. Then you can calculate your Ri.

Let's do this for glucose, we are going to determine the glucose rate RS.

First set up a balance for glucose. Shown here. Here you see what we need to measure, so let's do this.

Our system is defined by this continuous fermenter.

Here we grow our microorganisms. There is a nutrient flow in of 2 m3/h with a substrate concentration of 1000 mol glucose/m3. The outflow is 2 m3/h and has a substrate concentration of 1 mol glucose/m3 . The volume of the vessel VL is 100 m3 and the concentration glucose in the fermenter is 1 mol/m3. Here we have our substrate balance again. We'll assume that the fermenter is operating under steady state, again; meaning, there is no accumulation or depletion of substrate because concentrations and the broth volume are constant in time. So the accumulation term will be zero . Let's rewrite this and we get this . We know the flows with the concentration. And now we can easily calculate our substrate rate, Rs = -1998 moles/h. Note that it is negative because it is consumed . And such a rate can be calculated for every compound from its own compound balance.

Let's now calculate the rate, Rp, for our product PDO. First we set up the PDO balance in broth, shown here. When you look at this balance you determine what you need to measure.

Here is our fermenter again , The fermenter is operating in steady state, so the accumulation term is zero. And after rewriting the balance you can calculate your Rp and is 1000 moles/h.

Now that we have our substrate and product rates can we answer the money question?

When setting up a compound balance you are making certain assumptions. For example, the substrate you are using, the mechanism operating on the substrate is the consumption by the microorganism. You assume that all the substrate will be eaten by the microorganism. This could be true, but what if your substrate or product is unstable and is broken down into smaller molecules in the broth? This means another mechanism is acting on your substrate or product.

Another example is when your substrate or product is volatile, meaning some of your substrate or product will escape to the gas and is not eaten or produced by the microorganism, so another mechanism is acting on your substrate or product. When you don't know these hidden mechanism acting on your compound, you will get wrong rates. So we cannot answer the money question yet, we don't have the complete picture.

So how would you check if your substrate or product is volatile or not?

Well you have to measure the amount of evaporated substrate or product in the gas phase. To quantify the evaporation rate another balance needs to be set up, which is the gas phase balance. I'll tell you how to do this next time.