## IB01x - 4.7 - Basic approach to design and optimize a PDO fermentation process

In this unit we will summarize what we have learnt in the previous units this week. We will clarify how this knowledge will help you for the design and optimization of the large-scale bioreactor.

In any large-scale process design, we will start with the desired product, the selected feedstock and a need to produce a certain amount of product, in kilotonnes per year or tonnes per hour.

First of all we will evaluate the process reaction based on the black box model, as has been explained in week 3 of this course. Secondly, we will choose a bioreactor type (STR, BC or ALR), and mode of operation (batch, continuous, fed-batch). As a third step, the rate-limiting transport factor will be identified. This determines the size and geometry of the large scale bioreactor as well as the utilities, for example the size of the gas compressor, the impeller power demand, the cooling water pump capacity and the installed cooling area.

Let's bring back into your memory again the 4 rate-limiting transport steps that you may have in the large bioreactor: oxygen transfer – resulting in dissolved oxygen concentration gradients, carbon dioxide removal - causing inhibition of reactions, heat transfer – causing temperature gradients, and mixing – resulting in concentration gradients of the limiting substrate, but also pH and other compounds that are added to the bioreactor. Here we will provide the integrated picture, and the impact of scale.

The 1,3-propanediol case, produced from glucose in a large-scale aerated bubble column under continuous operation, gave for the 4 transport steps the following numbers: The required average transport rate of oxygen from the supplied air is 193 mol/$m^3$ h. Note that we calculated with our first design based on the geometry, pressure and total power input of the gas, that the actual oxygen transfer rate is only 135 mol/ $m^3$ h. This means that the design is not sufficient and needs to be improved. The concentration of $CO_2$ in the liquid phase, is about 7 mol/ $m^3$, set by the partial pressure in the gas phase and the gas flow rate. We calculated a cooling water flow rate of 2.46 $m^3$/s , needed to absorb the total heat produced by the process. And the mixing time was calculated around 69 s.

Before to make the mutual comparison, I will first highlight a factor that you frequently encounter in your process but is something that you should try to avoid as much as possible, that is: highly viscous broths, caused by filamentous microorganisms in combination with high cell concentrations.

In this video you can see how such viscous broth could look like. High viscosity restricts the motion of gas and liquid in the bioreactor and results in slowing down of the transport rates, and also the bioreactor productivity. Easily it can be a factor 10 and sometimes even 100. My message here is: avoid this kind of behavior, and nowadays this can be well realized via a combination of a good microbial host (for example yeast or bacteria instead of filamentous hyphae), strain modification and recipes with a low biomass concentration.

The design calculations show that to produce 100 kilotonnes of PDO per year, under optimal conditions the specific growth rate is 0.0245 $h^{-1}$, you need a broth mass of 2250 tonnes, in a fermenter with a liquid height of 25 m and 10.7 m wide. The key performance indicators, which strongly determine the economy, also follow:

- The PDO titer is 228 kg/tonne
- The productivity is 5.6 kg PDO/tonne broth/h
- The yield of PDO on sugar is 0.33 kg/kg, which is equivalent to 63% of the theoretical maximum

So far, we showed averaged numbers for the whole fermenter. But, when you zoom in on the fermenter content, then local differences become visible. In the environment of the cells, there are concentration and temperature gradients.

Because you feed glucose at a certain point in the reactor, and the fact that the mixing time is larger than zero, this means that a glucose gradient exists in the reactor. Also, a gradient of dissolved oxygen concentration exists due to the high hydrostatic pressure and the higher oxygen concentration near the gas inflow.

A glucose concentration gradient is found ranging in this case, from 0.45 mol/$m^3$ close to the glucose feed inlet, gradually decreasing when you move away from the inlet, dropping to about 0.085 mol/ $m^3$ at the far end of the tank. This is a span of a factor 5. A second gradient is for the oxygen transfer rate: at the bottom it is high due to a high hydrostatic pressure and high oxygen concentration in the gas phase, while close to the gas outlet the overall pressure is lower and also the partial pressure is reduced due to depletion of the gas phase. As a consequence, there are also dissolved oxygen concentration gradients. A third aspect is the dissolved CO2 gradient: this appears to be relatively modest, ranging between 6 and 7 mol/ $m^3$. Still this can be critical when you operate near the inhibition limit. Finally , the cells move, on average once every 15 minutes, through the external cooling loop, and then they shortly undergo a cold shock of 5 degrees before returning to the bulk of the tank. Altogether, the cells encounter all these changes at the same time and this has an impact on their performance. A conclusion that we draw here is that the microorganism should be very robust! It becomes even more critical when it is recognized that the conditions for individual cells may differ a lot as well.

Here the life-line of one possible individual cell is shown. It is exposed to severe concentration fluctuations, and in the given time frame runs one time through the cooling loop. Other cells can stay long in one zone with low or high glucose and oxygen levels, while others may move back and forth very fast. For temperature, some cells may undergo rapid T changes, much faster than once in every 15 minutes, while others may observe constant T for hours. As a result small cell sub-populations may develop with extremely altered performance, sometimes affecting the whole population for example when they are dead and undergo lysis.

Dealing with such dynamics is the core for scale-up research. But the right direction is not from small to large scale, but rather the reverse: from large to small scale. This is called scale-down. In a scale-down study, the conditions in the large bioreactor are as precisely as possible mimicked in a smaller set-up, that can be operated at low cost and high-throughput in the lab, in a so-called scale-down simulator. After the experiments are completed, findings and optimization leads then can be implemented at reduced risk in the factory.

The final aim is to create similar conditions in the lab as on large scale so that the cells do not see any difference. Here 3 examples of scale-down simulators are presented: Two interconnected stirred tanks, with feeding or aeration in only one of the 2 compartments. One stirred tank plus one plug flow unit, with or without liquid dispersion. Glucose feed can be at the entrance of the plug flow unit to set a concentration gradient. One stirred tank in which the conditions for all the cells are varied at the same time, for example via on-off feeding or aeration.

Considering the implementation stage, there are in general 3 ways to optimize your bioreactor process:

You may try to optimize your microbes. If you have filamentous organisms, then try to produce the product in a non-filamentous host organism. Another way is to select or, alternatively, construct more robust cells, tolerant to stress such as concentration and temperature changes. Physiology: try to better control critical process parameters, e.g. via other feed schemes, other feedstock properties and composition, recipes that give lower biomass levels, or other specific growth rates, etc. And then the category hardware adjustments: location and number of C-source inlets, stirrer type and configuration, gas flow rate/headspace pressure, design of internals, aspect ratio, etc.

In conclusion then, the following can be summarized.

A powerful design method has been presented and applied to the PDO case: Based on input on 4 transport mechanisms, in combination with constraints from metabolic stoichiometry and steady state compound balances, a design space is created with only 1 degree of freedom. This can be arbitrarily filled, but a useful input is the specific growth rate of the microorganisms. When this specific growth rate is fixed, then all outputs for design (such as fermenter size, G/L flow rates and composition, local conditions for the cells) as well as the optimal titer, yield and productivity are fixed. A cost model can then be used to find the optimum specific growth rate and all other related conditions.

And finally, I hope that I have made clear that the best rational optimization method is scale-down, rather than scale-up to expose the microorganism to the mimicked full scale environment, and identify improvement factors in the lab. So that completes this unit.

See you next one!