IB01x - 3.4 - A PDO black box model: experiments for parameter identification

Last week I introduced the framework of black box models, for two product categories: products which give the cell energy, leading to anaerobic processes, and products for which the cell needs to invest energy, which must be done aerobically.

This slide shows the Black Box models for both product categories. They are partly the same, for example the hyperbolic substrate uptake relation, and partly different, for example for the Herbert-Pirt relation, which contains 3 terms for the aerobic case and only 2 terms for the anaerobic case.

Also the qp (μ) relation is different, being non-linear for the aerobic case and linear for the anaerobic case.

Finally, it should be recognized that each model has only one free variable. It is most practical to choose μ as a free variable. In that case, for a selected μ -value, the qp(μ) relation gives you the qp. And because you have μ and qp that gives you qs through the Herbert-Pirt equation, and then the first equation gives you cs.

Let us now focus on the aerobic black box model. We can see that this model contains 7 parameters, which are here indicated in blue. To determine these parameters we need data obtained from experiments. When we look at the three black box model functions, it is obvious that for the hyperbolic substrate kinetics we need datasets of qs and cs, for the Herbert-Pirt relation, we need datasets of qs, μ and qp, and for the qp(μ) function we need datasets of μ and qp, The conclusion is that we need datasets of cs, qs, μ and qp to obtain the 7 parameters.

The experiments should then give you sets of cs, μ, qp and qs, which you can enter into these equations. In one of the previous lectures I already explained how you can obtain these q-rates using biomass, product and substrate balances, and which measurements have to be done in chemostat experiments.

Now the question is, how many chemostat experiments do I minimally need to perform to get the parameters?

You do not want to do too few experiments but you also don't want to do far too many.

Let's check the system of equations with the unknowns that we have in order to answer this question.

In the case of only one chemostat experiment, you get one set of qp, qs, μ and cs values. You enter the qs and cs values in the substrate uptake equation. What you see here now is that you have one equation with two unknowns, the parameters qs,max and Ks.

For the second relation, Herbert-Pirt, we fill in the qs, μ and qp values. You can see that you have one equation with three unknowns, which are the parameters a, b and ms. Then in the

third equation, the qp(μ) relation, we fill in the μ and qp and then you see that here you have one equation with two unknowns, the parameters α and β. So when you do one experiment it's clear that you cannot determine any of the 7 parameters, because there is too little information.

So you have to perform a second experiment this time with a different μ value, so you get a new set of values for cs, qs and qp.

So from experiment 2, you enter these values in the 3 black box model equations again and now you get a second system of three equations, which contains the 7 parameters.

Here you see that we can solve for the parameters of the first and the third equation because we have 2 equations of each, and in both equations there are two unknown parameters. However, we cannot solve the Herbert-Pirt equation because we have only 2 equations and 3 unknown parameters: a, b and ms. So two experiments is not enough to determine all parameters.

With three experiments you see that we have even too much information for the first and the third equation, so for the parameters of these equations you can now do non-linear regression.

The second equation can now also be solved because we have 3 equations with 3 unknowns. So we need to do at least 3 experiments at 3 different μ values to determine the parameters. In practice you have to do more experiments, because the experiments have measurement errors that propagate in the calculated q-values and in the parameter estimations. The easiest way to do experiments is for instance to perform 5 to 10 different chemostat experiments covering the range of low to high μ-values.

Here we have experimental data on 7 chemostat experiments with different μ values for the PDO producing organism. To determine the Ks and qs,max values, you can plot the qs versus cs values.

You can of course perform a non-linear regression, but it's always good to visually check the computer output. So what you can easily see here is that the dotted line equals the qs,max. And then you know that the Ks value is the substrate concentration at half qs,max.

So this is a way to quickly estimate Ks and the qs,max . Then you can use a non-linear regression programme to produce these numbers with the error on each parameter value.

It is also important to pay attention to the units of the parameters as indicated.

For the qp versus μ function you can also plot your experimental results. In this case you don't know the exact shape of the graph. Previously I assumed that it would be hyperbolic, but it can have different kinds of shapes.

So in the end you need to fit the data to a mathematical form that comes closest to the qp and μ data . In our case this is a hyperbolic function, and you can determine the parameters α and β of your qp (μ) function.

And then finally we have the Herbert Pirt substrate distribution relation.

For our aerobic, energy consuming product the Herbert-Pirt equation has 3 terms. So there are three parameters and you see the equation is linear in the 3 parameters a, b and ms.

This tells you that this is a linear regression problem which is easily performed, leading to the parameter values shown.

Note the parameter dimensions, which are very important to understand.

Summarizing, to obtain the parameters of an aerobic BB model, you need at least 3 chemostat experiments at different μ values. And when you have your data sets of cs, μ, qs and qp it's always good to do a graphical evaluation of your results and then use the standard computer techniques to get the relevant parameter values. You should note that it is also possible to do other experiments, for example fed-batch which in one experiment gives sets of cs, μ, qs, qp values covering a large range of μ-values.

The estimation of the black box model parameters is then mathematically more complex, but follows the same principles as outlined before.

See you in the next unit!