Jo Maertens

CFD study of the metabolic response to fast changing substrate concentrations due to spatial heterogeneity in industrial fermentors

Thesis submitted in fulfilment of the requirements for the degree of Doctor (Ph.D.) in Applied Biological Sciences.

Summary

Scaling-up fermentation processes from laboratory-scale conditions to large-scale conditions generally results in a reduction of the overall process yield and productivity. This due to the interplay of biological, chemical, and physical factors. In this work, different tools have been developed and applied which may help to elucidate the mechanisms causing this generally observed yield reduction.

Then, tools to describe micro-organisms in detail are necessary. Hence, the state of the art approaches for metabolic modelling, typically used in the domain of metabolic engineering, were reviewed. The strategy to be followed for optimising a production host for overproducing a target compound should predominantly depend on its characteristic properties. In this respect, issues like the dependence of the target compound's synthesis on severe (redox) constraints, the characteristics of its formation pathway, and the achievable/desired flux towards the target compound should play a role when choosing the optimisation strategy. Still, due to the vast variety of biochemical pathways and the lack of extensive data sets the usefulness of these mathematical techniques remains limited. In this Ph.D. study some of the reviewed methods have been applied, such as partial least squares, approximative metabolic modelling, and cybernetic modelling.

The usefulness of partial least squares regression has been demonstrated using elementary flux mode data. It was possible to rapidly pinpoint potential targets for modification of the microbial production of succinate by *Escherichia coli*, without the need for experimental data. The identified targets are in agreement with the literature data (modification of the expression of these genes proved to be beneficial to increase succinate yield). This approach has therefore passed a first validation round. Further evaluation is however needed.

Conversely, a dynamic model-based approach focusses on the identification of the flux controlling reactions, which are targets for genetic modifications. In view of decision-making in metabolic engineering, it is important to assess the uncertainty on the calculated flux control coefficients. Both an uncertain model structure and uncertain parameter estimates can be the cause for the overall prediction uncertainty. For an illustrative pathway this uncertainty has been properly assessed. Multiple approximative kinetic formats have been used to identify the flux control coefficients of the small network model studied. It has been shown that the applied model structure significantly influences the distribution of the flux control coefficients.

Micro-organisms in large-scale bioreactors are characterised by a particular metabolomic and proteomic make-up, which allows maximisation of their growth under those conditions, *e.g.*, mixed acid fermentation and overflow metabolism. Since this complies well with the idea behind cybernetic modelling, cybernetic models were finally retained to describe the biophase in large-scale bioreactors. The rationale of the cybernetic school of thought is that micro-organisms are believed to optimise their behaviour, *e.g.*, with respect to growth or substrate uptake. This is achieved by allocating, by means of a controller, the limited resources a micro-organism disposes of to these enzymes yielding the optimal performance. In spite of recent efforts to increase the robustness of the approach, *e.g.*, by introducing elementary flux modes as intermediate level of control, there still remain some issues unresolved. For instance, several rival control laws for enzyme activity have been derived. These rival control laws had a different no-cost activity and are based on the fact that mechanisms have been reported in the literature for both the activation and inactivation of enzymes, which may have a cost. However, due the lack of appropriate data it was not possible to distinguish between those rival control laws.

Subsequently, set-ups are discussed which may help to gather the necessary data to experimentally study microbial metabolism and to gather the necessary data with a view to parameter identification and model structure identification. To this end, a modus operandi of the Bioscope is proposed to study microbial oscillating systems. A strategy has been proposed to control the opening and closing of the sample ports, so that this equipment can also be used to collect the samples from multiple perturbation experiments, without perturbing the microbial oscillating culture from which the cells are taken.

A strategy to design a scaled-down reactor is outlined as well. The innovative aspect

of the presented approach is that it attempts to mimic the environmental conditions observed by the micro-organisms, by making use of computational fluid dynamics simulation results, rather than to focus on macroscopic variables, such as circulation time and mixing time, as those macroscopic variables are far from ideal to be correlated with degrees of conversion. Such scaled-down reactors allow to mimic on a laboratory-scale, the large-scale conditions in an attempt to anticipate the outcome on a large-scale. The proposed controlled set-up, a controlled system consisting of two continuous stirred-tank reactors in a loop, allows to imitate similar conditions as those that occur in large-scale bioreactors. To reduce the control efforts one could use a maximal value for the substrate concentration set points, since the cellular response to environmental concentrations much larger than the affinity constant becomes saturated.

Finally, a method has been proposed to use segregated models, in which micro-organisms are not considered identical, and in which the cells are structured, *i.e.*, the internal composition and structure of the micro-organisms is considered, to describe the biophase in large-scale bioreactors using computational fluid dynamics. The description of the biophase in a Lagrangian way, *i.e.*, following the cell's path through the reactor, is an obvious choice since the behaviour of a micro-organism is determined both by the reigning environmental conditions and its intracellular make-up. This intracellular make-up is expected not to be identical for all micro-organisms, due to the stochastic nature of particle transport and the fast metabolic response to the observed fast changing environmental conditions. Such an approach is computationally quite demanding because every micro-organism is linked to a set of differential equations. However, by considering that the overall picture is merely the result of all individual micro-organisms it is only needed to track a limited number of particles in order to obtain a good idea of the consumption and production of metabolites throughout the large-scale bioreactor. Indeed, the dynamics of the overall system can be captured by averaging out the behaviour of this limited number of particles over the whole population, hereby making use of prior knowledge about the microbial behaviour.