Gourmet Mix for Bacteria

When optimizing a process, nothing should be left to chance, not even the design of the laboratory experiments. By applying this idea, the yield of a biosynthesis process can be tripled with little effort.

When dealing with biosyntheses, biotechnologists are often confronted with the task of optimizing the composition of the supporting medium. Once some promising substances have been chosen, the question of their mixture ratios needs to be addressed. In such situations, experiments are often performed by varying only one factor of influence upon changing from one test to the next one. The main disadvantage of this approach is obvious: the experiments may be inappropriately distributed within the whole experimental region, so that no really good configuration can be found.

**Much information, few experiments**

The aim of gaining a maximum of information out of a minimum of experiments can be achieved with Statistical Design of Experiments. The experiments to be carried out then result from the specification of the response variables, which are to be optimized, and of the factors of influence. In the following case study that concerns the optimization of a biosynthesis process, the titre of the metabolite produced should be maximized by varying the components of the medium. This medium consists of the following substances, whereby their concentration levels may be varied within predefined bounds:

- saccharose (from 20 to 50 g/l)
- protein source (from 10 to 50 g/l)
- cottonseed flour (from 0 to 20 g/l)
- calcium carbonate (from 0 to 5 g/l)
- yeast extract (from 0 to 10 g/l)

With only these five factors of influence, the situation is already hardly manageable. Consequently, the use of a software package which safely guides the user through the different steps of the experimental design process, proves very helpful.

In general, the experimental design process decomposes into three phases: screening, modelling and optimization. In the first phase, there are many potentially important factors, whose true effect is mostly unknown. Therefore, the most important ones are determined and those which are likely to be unimportant are eliminated. The modelling phase then follows, in which the factors that have previously been identified as important get examined more closely and the number of factors that really have an impact gets reduced. The last phase, the optimization phase, aims at representing as accurately as possible the relationship between the most relevant factors and the response variables.

**Flexible application of STAVEX**

The present experimental design allows the user to start with the optimization phase because only five possible factors of influence are available. The ranges of variation of the factors and the properties of the response variable now have to be entered into STAVEX. The software then suggests different designs of experiments. These are generated in such a way that a maximum of information can be extracted from the results of a small number of experiments. The user may also modify the designs proposed or even define plans himself and then use the software only for data analysis purposes. Once a so-called vertex-centroid design has been chosen, 26 experiments have to be carried out and the titre measured each time has to be entered into the software.

The analysis shows a good model fit. The titre of the metabolite is maximal for the following constellation: saccharose: 50 g/l, protein source: 50 g/l, cottonseed flour: 20 g/l, calcium carbonate: 0 g/l and yeast extract: 0 g/l. The first three factors are at the upper limit of their range of variation, whereas the last two factors are at their lower limit 0. In order to achieve the maximum titre, neither calcium carbonate nor yield extract should be included in the composition. The step to optimization can now be repeated with this increased knowledge about the situation. Both factors calcium carbonate and yield extract are now left out. Moreover, the range of variation will be adapted by raising the upper limit of the remaining factors saccharose (from 50 to 80 g/l), protein source (from 50 to 80 g/l) and cottonseed flour (from 20 to 60 g/l).

The software now suggests to the user a Box-Behnken design with 13 runs. These experiments are performed, recorded and analysed. In order to enable an easy interpretation of the results, STAVEX offers a broad range of graphics. A better model fit results from the new analysis and the optimal point now lies inside the analysed experimental area (saccharose: 48.53 g/l, protein source: 51.32 g/l, cottonseed flour: 38.38 g/l). The titre of the metabolite amounts to 195.5 mg/l and the 90% confidence interval is [176.6, 214.5].

Since a statistical model can only represent reality in a simplified way, it is necessary to examine the fit and the
model quality. From this point of view, a very good result has been achieved ($R^2 = 0.97$).

Only 40 experiments for a triple yield

Additionally, it is recommended to run a further, so-called confirmation experiment with the optimal parameter setting (saccharose: 48.53 g/l, protein source: 51.32 g/l, cottonseed flour: 38.38 g/l). The titre measured in this experiment amounts to 205 g/l, which highlights the excellence of the model. Altogether, only 40 experiments were needed to triple the titre of the very first experiments, which was 60 mg/l.

Statistical Design of Experiments

More than 200 companies from various branches, predominantly the pharmaceutical, biotechnological and chemical industries, as well as universities and research institutes currently use STAVEX all over the world. The software is equally well suited for the optimization of processes and of formulations and proves very helpful to derive the Design Space in the Quality by Design context.

STAVEX has for example been applied to the following projects:

– Optimisation of the composition of culture media in biotechnology
– Parameter optimisation during the scale-up of synthesis processes (both for chemicals and API's)
– Analysis of the dissolution speed of sustained-release formulations
– Optimisation of tablet formulations
– Thermo-sealing of blister packages for tablets
– Optimisation of the formulation of lacquers subject to complex restrictions
– Optimisation of ceramics mixtures
– Optimisation of additive mixtures to improve the properties of spray concrete.

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