HYBRID MODELLING FOR ON-LINE PENICILLIN FERMENTATION OPTIMISATION


Abstract: This paper describes the procedures that are necessary to arrive at a model that is sufficiently accurate to be used in an on-line penicillin fermentation optimisation scheme. A structured mechanistic model developed previously was available but this model failed to account for the effects of low levels of dissolved oxygen on growth and production. When moving towards optimising the fermentation, dissolved oxygen becomes the parameter limiting process productivity and hence it is important to be able to account for its process influence. Terms predicting dissolved oxygen changes and describing its effect when at low levels were included in the model to compensate for the reduction in growth and production. Even so, the natural variation experienced in the fermentation resulted in process/model mismatch. On line correction of model coefficients via an observer approach provided the accuracy required for optimisation purposes. The improvements in the accuracy of the model predictions are demonstrated.

Keywords: Fed-batch fermentation, hybrid modelling, artificial neural networks, penicillin fermentation

1. INTRODUCTION

Bioprocess engineers have strived for many years to produce accurate models describing fermentation process behaviour. The driving force behind this is that once a representative model is built, it can be used in fault diagnosis, performance estimation and prediction, scheduling and optimisation. The work described in this paper concentrates on optimisation specifically, with particular application to a fed-batch penicillin fermentation.

In batch and fed-batch bioprocesses, the maximum performance is achieved by optimising the initial conditions and subsequent profiles of manipulated variables such as feed rates, temperature and pH during the process operation. However, the industrial use of models for optimisation purposes has been limited, with a more heuristic procedure often being preferred. Although this has resulted in considerable improvements in performance, the application of modern mathematical optimisation theory offers the potential of even greater benefits. Classical approaches to solving the optimisation problem exist such as Pontryagin’s Maximum Principle. An overview of the topic is given in Constantinides (1979). Alternatively, to calculate the optimal profiles during the batch and fed-batch mode of operation, a pre-defined function such as polynomial or more complicated shapes can be used to describe the variations in the manipulated variables. Once parameterised, various optimisation methods can be applied to find the coefficients of the pre-defined function. Further details of this approach can be found in Rodrigues and Filho (1996) and Montague and Ward (1994).

At the heart of the optimisation algorithm is a model of the process to be optimised. Although there are many subtle variations in model form, in general models belong to one of two categories: mechanistic or empirical. Empirical models are generally structured and do not take account of the microbiological behaviour from a fundamental understanding perspective. Mechanistic models, on the other hand, attempt to approximate these characteristics in mathematical form. Since mechanistic models capture physical behaviour, they have the potential to be more precise than empirical models. This is particularly the case when considering extrapolation beyond the regions for which the model was constructed.
Numerous mechanistic models have been published to date describing the penicillin fermentation (Heijnen et al., 1979; Bajpai and Reus, 1981; Nicolai et al., 1991). However, when industrial application is considered, it is generally found that existing model structures fail to take into account important issues. For instance, the variation in environmental conditions in large-scale operation can be difficult to model with any degree of certainty. An alternative approach is to use empirical models. Many forms of empirical model have been applied to bioprocesses. These range from linear (Linko et al., 1992) to non-linear (Montague et al., 1992; Warnes et al., 1996). While success with these techniques has been reported, they do have major drawbacks. Namely, the validity of these models outside the region of the training data is limited. Ideally what is required is a model which possesses the advantages of both the mechanistic and empirical approaches. The hybrid modelling concept offers this potential.

As with all modelling techniques there are a number of options available. One approach is to couple artificial neural networks (ANNs), an empirical modelling technique, with a mechanistic model. Fundamentally, there are two main strategies: serial and parallel. Psichogios and Ungar (1992) proposed the serial approach to model batch and fed-batch fermentation processes. The ANN was used to model variations in the specific growth rate, which was then utilised as a parameter in the mechanistic model. This approach has also been used to derive a model for fed-batch production of a recombinant protein from mammalian cells (Dors et al., 1995). Su et al. (1992) proposed a parallel hybrid method where the ANNs compensate for the difference between the mechanistic model and the real process. Thompson and Kramer (1994) proposed a hybrid model that combines the serial and parallel approach. They illustrated the methodology by predicting the biomass and product concentration for a simulated penicillin process. The integration of ANNs with mechanistic models has also been applied to improve the prediction accuracy of the state variables of mammalian cull cultures by Fu and Barford (1996). De Azevedo et al. (1997) compared a hybrid model, consisting of an ANN and mechanistic model, with the prediction capabilities of the individual models for fed-batch yeast fermentation. Their investigation demonstrated that the hybrid model proved to be more effective. ANNs are not the only model form used to enhance mechanistic model predictions. For instance, Schubert et al. (1994) developed a hybrid model of fed-batch yeast fermentation by integrating a variety of modelling techniques such as: ANN, mechanistic model and heuristic knowledge using fuzzy rules. Johansen and Foss (1997) proposed a framework that combines different kinds of local empirical and mechanistic models into a global model.

Whatever optimisation strategy adopted it is essential in the first instance to have a model for the optimiser to utilise. It is not the objective of this paper to describe the use of the model together with the optimisation scheme. Space precludes coverage of both modelling and optimisation strategies. Fundamental to the success of the optimiser is the model. This paper therefore concentrates upon obtaining a model of the penicillin fermentation that has sufficient accuracy to be used as the basis for optimisation.

2. EXPERIMENTAL SYSTEM

An industrial strain of *Penicillium chrysogenum* grown in a 5L bioreactor in the Centre for Bioprocess Engineering at Birmingham (as described in Paul et al., 1998), was used for experimental trials. Eight fed-batch penicillin fermentations were carried out using a range of fixed and variable feed profiles with the standard on- and off-line variables monitored. Additionally, product and residual glucose concentrations were made available using an on-line HPLC system, and off-gases were analysed using a mass spectrometer. To further enhance the monitoring, off-line image analysis provided information on the physiological state of the culture. The process information was monitored and the manipulated variables controlled by a SETCIM control system (AspenTech, Inc.). An Internet link using SequeLink Connect Administrative Tool was set up between SETCIM in Birmingham and a monitoring system at Newcastle University. The link allowed all the SETCIM data to be collected, saved and used in real time at Newcastle and information ascertained from the model returned to Birmingham.

3. MECHANISTIC MODEL UPDATING

Paul et al. (1998) developed a comprehensive structured model describing the behaviour of the penicillin fermentation. Verification against experimental results demonstrated that the model gave good predictions of fermentation behaviour but only when the operating policy was to set carbon feeds at such a level as to maintain levels of dissolved oxygen (DO2) higher than a critical value. Unfortunately such conditions may not prevail when optimisation of the fermentation is considered. When optimising the fermentation process it is the DO2 levels that form the constraint that the optimiser pushes the process towards. Put simply, high levels of active biomass means more penicillin producing cells. However, too high a biomass concentration leads to mass transfer limitations and a resulting fall in DO2. In this case as DO2 falls firstly penicillin production becomes inhibited and if it falls further active biomass is destroyed. Ideal conditions are therefore high biomass just avoiding the effects of DO2 limitation. These effects are confirmed by the studies of Henriksen et al. (1997). They studied the influence of the DO2 concentration on penicillin biosynthesis in steady-state continuous cultures. They showed that at low DO2 concentration, the specific penicillin productivity decreases and further lowering the DO2
concentration results in loss of product. However, they claimed that the penicillin productivity was instantly recovered to its maximum value when the DO₂ concentration was reset to a value above the limited DO₂ values. In their case, levels of DO₂ critical to biomass were not reached.

Unfortunately, the structured model developed by Paul and Thomas (1998) does not contain terms describing the influence of the DO₂ on the biomass and product formation. From the considerations above it is clear that such terms are vital for optimisation purposes. The DO₂ influence on biomass and product concentration can be incorporated within the mechanistic structured model using the following relations (equation 1) (Paul (1999)):

\[
DO_x = \frac{1}{1 + \left(\frac{K_x}{DO_x}\right)^{N_x}} \\
DO_p = \frac{1}{1 + \left(\frac{K_p}{DO_p}\right)^{N_p}}
\]

The DOₓ term (eqn. 1) was used to modify the rate expression for branch formation (\(r_{b,o}^1\)) and extension rate (\(r_{e,1}^1\)), while the DOₚ term (eqn. 2) was used to modify the rate of product formation (\(r_p^1\)) in the equations given in Paul et al. (1998) in the following manner:

\[
r_{b,o} = DO_x \cdot r_{b,o}^1 \\
r_{e,1} = DO_x \cdot r_{e,1}^1 \\
r_p = DO_p \cdot r_p^1
\]

In this application study, only the DO₂ influence on product formation was considered, so the parameter \(K_x\) was equal to 0 and \(N_x\) to 1. The reason why this is the case comes from a fundamental appreciation of the process. Significant loss in penicillin production occurs before cell growth becomes affected to any extent by low levels of DO₂. The optimum conditions for operation are obviously where penicillin production is not significantly inhibited. Therefore the region over which the model is required to be accurate does not stretch into regions where DOₓ would be significantly different from unity. The parameters \(K_p\) and \(N_p\) were obtained using real experiment data (Fermentation 6 in Fig. 3) and the simulations using the structured model without the DO₂ limitation. The production rate \(dp/dt\) was calculated from the experimental data, while the maximum production rate \(dp_{max}/dt\) was obtained from the model simulations. The ratio between these two rates (\(dp/dt\) vs \(dp_{max}/dt\)) was then plotted against the DO₂ data (given as circles in Fig. 1). The parameters \(K_p\) and \(N_p\) (eqn. 2) were optimised using this information and non-linear least squares. The optimised parameters are \(K_p\) = 7.03 and \(N_p\) = 3.93. The estimated relation was plotted as a solid line in Fig. 1.

**4. HYBRID MODEL DEVELOPMENT**

The mechanistic model modified to take into account low DO₂ levels is not suitable for use in an optimisation scheme. DO₂ varies primarily as a result of carbon feedrate variation. An optimisation scheme requires a model that when feed profiles are specified is able to predict penicillin production levels. DO₂ is therefore a model output, although it obviously influences other states.

A serial hybrid model, consisting of a feedforward artificial neural network (FANN) estimating DO₂ values and the structured model including the DO₂ influence on the product formation, is proposed in 2. This structure can predict the product concentration by taking into the account the DO₂ influence.

![Fig. 2: Proposed hybrid model structure](image)

The FANN was developed off-line using the experimental data presented in Fig. 3. The inputs to the FANN were the feed, total feed, volume and the batch age. From the total of eight fermentations (Fig. 3), four
Fig. 3: Fermentations used for this application study

Fig. 4: Actual and estimated DO₂ vs. Sample Number
fermentations were used for training (Fermentations 4, 5, 6 and 8) and the remaining four for testing (Fermentations 1, 2, 3 and 7). The batches to be used for training / testing were selected in order to get wide model coverage. The optimal topology was 3 nodes in a single hidden layer and was determined by carrying out multiple training runs. The actual and estimated DO2 measurements for the training and testing data are presented in Fig. 4.

5. MODEL / PROCESS MISMATCH CORRECTION

If the above model were used in a real-time optimisation scheme then any discrepancies between the process and the model would lead to non-optimal behaviour. Although reasonably accurate in its present form, improvements can be made. In particular, during the fermentation run the on-line information can be utilised to update the model, and subsequently the updated model can be used to re-optimise the feed in real time. The model can be updated using parameter re-optimisation of the hybrid model (either the mechanistic or ANN model). However, this is not practical to implement in a real-time environment. Apart from the time involved, selection of appropriate data for model building requires manual intervention. As an alternative, only a few parameters could be updated and in this case, the specific production rate ($\mu_p$) was chosen. $\mu_p$ has a direct and significant effect on the penicillin production rate.

Fig. 5 presents the proposed scheme for the on-line model correction procedure. It also shows how the model sits within the optimisation algorithm producing carbon feed profiles.

Fig. 5 - On-line model correction and its place within the optimisation scheme

In the figure, the discrepancy between the hybrid model prediction and the real data is removed by feedback correction following the observer predictor / corrector concept. The error between the actual (P) and predicted product concentration ($P_{\text{mod}}$) is firstly determined. In this case an on-line HPLC supplied frequent measurements of penicillin concentration. The error was used to adjust $\mu_p$ using PI feedback correction. In general observer speed of response is a balance between tracking error and amplification of noise. The observer gains and the sampling interval in this case were obtained empirically using the experimental data so that an acceptable tracking of product concentration was obtained without making changes that were too significant to $\mu_p$.

Following off-line algorithm adjustments, the algorithm was implemented on-line. Results of the first trial are presented below in Figs. 6 and 7.

Fig. 6 - Variations in corrected $\mu_p$ over the fermentation

Fig. 6 shows that no model correction takes place until twenty five hours into the batch. The reason for this is that no penicillin is produced until after this time. Following that some severe changes are made in $\mu_p$ (denoted by the continuous line in Fig 6). These are as a result of the observer responding to measurement noise. This indicates that a reduced observer gain is required when the model is implemented within the optimisation scheme. The improvements possible with this reduced gain are indicated by * in the Figure.

Fig. 7 - Comparison of model prediction and measured penicillin concentration

Fig. 7 shows that there is very close agreement between measured and model predicted profile. The dashed line in Fig. 7 shows the penicillin prediction resulting from the model without $\mu_p$ correction. The continuous line indicates the $\mu_p$ updated penicillin prediction with the reduced gain and * indicates the on-line HPLC measurements.

6. CONCLUDING COMMENTS

In this paper a mechanistic model of penicillin fermentation was taken as the basis for a hybrid model.
Limitations of the mechanistic model were overcome by making use of an artificial neural network. The resulting hybrid model although improved, still lacked the precision necessary for process optimisation. This process / model mismatch resulted from natural variability. To overcome this, a 'predictor / corrector' method was used to modify a key model parameter which allowed the production of penicillin to be tracked with higher accuracy. The on-line model modification now provides a model of sufficient accuracy for optimisation purposes. If further experiments to verify the optimisation strategy reveal similar µ trends then a prespecified typical profile would be more appropriate for model use therefore requiring more limited off-line correction. Further discussion of the optimisation approach that this model is embedded within can be found in Ignova et al (2001). A final comment to make concerns model updating; here, an on-line HPLC was available to provide frequent measurements of penicillin concentration. Such instruments will not always be available. In these cases the use of other on-line measurements could be considered for on-line correction given that observability type assessments are undertaken.

ACKNOWLEDGEMENTS

The authors of this paper would like to acknowledge the financial support of the UK Biotechnology and Biological Sciences Research Council.

REFERENCES


Paul G.C. (1999). Personal communication


