Prediction of *Pseudomonas putida* growth during batch fermentation by controlling acid production rate

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Abstract: For batch fermentation with constitutive promoter the concentration of recombinant protein is often highest at the same time when the absolute growth rate achieves maximum. Hence during fermentation it is important to detect the moment of the maximum microbial growth rate. Accuracy of direct observation is limited by time interval between two subsequent measurements. For more exact estimation, extrapolation is required. We tried to compare the ability of popular models for prediction of the growth of *Pseudomonas putida* recombinant strain during fermentation. On the other hand, we took into account some additional information for the accuracy of prediction. The suggested model additionally uses the information from the fermenter pH stabilisation system. Experimental testing shows that this allows for prediction of the growth rate more exactly than by using the classic models of growth kinetics, resulting in the higher final amount of the recombinant protein.

Key words: *Pseudomonas putida*, fermentation, pH, growth prediction, mathematical modelling.

Introduction

Biosynthesis of recombinant proteins during fermentation is not necessarily related to the growth of biomass or primary metabolism. It is commonly supposed that for batch fermentation with constitutive promoter the concentration of recombinant protein is highest at the same time when the absolute growth rate achieves maximum. Hence, during the fermentation it is important to monitor the growth rate, detecting the time, when it stops increasing and starts declining. The operator usually performs 2–3 measurements of cell optical density per hour. It is thus desirable to establish the moment of maximal growth rate with the higher precision than the time interval between two optical density subsequent measurements of cell suspension.

Most of the mathematical models of microbial growth have certain potential to extrapolate, predicting the growth rate in near future. The quality of extrapolation varies from model to model, not necessarily coinciding with ability to fit experimental data (a good example is a polynomial regression of high degree). One of the tasks of this work was to compare the ability of popular models for the prediction of the growth of *Pseudomonas putida* recombinant strain during fermentation. The second idea was that the accuracy of prediction can be increased by taking into account some additional information.

Some parameters are measured much more frequently than optical density of cell suspension, not requiring extrapolation to obtain the current value. Such information could be used inside the model to improve the prediction of the growth rate. The parameters involved into control loops such as temperature, pH or pO_2 remain inside certain narrow interval and cannot provide much information about development of the current fermentation process. However, the proton production rate (qH^+) is quite often directly related to the growth (Roos & Luckner, 1984; Huth et al., 1990). We can find qH^+ from the fermenter information about the addition of pH control reagents. In the simplest case a fixed stoichiometric relationship between qH^+ and the growth can be determined for a certain substrate employed (Castrillo et al., 1995) and the growth rate can be calculated by multiplying qH^+ by a certain determined coefficient (Vicente et al., 1998).

The current work deals with more complicated situation: while qH^+ and the growth rate remain still related, the proportionality coefficient changes in time. This is the most likely if the medium contains multiple substrates, e.g. yeast extract. Switching from glucose to some energy source requiring deamination of amino

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