

## Real time optimization of fed-batch culture of recombinant yeast

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**Abstract**

A real time optimization algorithm for fed-batch cultures of recombinant yeast to determine the optimal substrate feed rate profile has been developed. Its development involved four key steps: (1) development of reliable adaptive model, (2) development of optimization algorithm, (3) design of on-line model update algorithm to be incorporated into the optimization algorithm and (4) experimental validation. A recombinant *Saccharomyces cerevisiae* producing human parathyroid hormone (hPTH) was chosen as the model strain. It was found to be very successful in maintaining cell growth and galactose consumption at high levels, thus resulting in significant improvements in the productivity (up to 2.1 times) and intact hPTH concentration (up to 1.5 times) compared with the case of an intermittent glucose and galactose, or galactose feeding.

**Introduction**

Development of high-performance systems for the control of fermentation processes able to handle intelligently various situations has been a long-cherished ambition of bioprocess engineers. Traditionally, this has been pursued by the application of complex mathematical methods and conventional control theories. However, these approaches in this field could not guarantee fully successful application due to their narrow validity and large computational burden originated from the great complexity and uncertainty of fermentation process. The need for better control has led to the search for new methods capable of analysis of complex biological plant and treating various disturbances and uncertainties. The major goal of the present study is to develop an efficient method based on artificial intelligence techniques for the maximization of foreign protein production in fed-batch cultures, which has a good adaptability and requires only little a-priori knowledge on the system to be handled.

## Materials and methods

A human parathyroid hormone producing recombinant yeast, *Saccharomyces cerevisiae* L3262 was grown aerobically. Two hundred milliliters of seed culture was transferred to a 5 L jar fermentor containing 1800 mL of start-up medium. When glucose was exhausted and the dissolved oxygen concentration began to increase rapidly, a concentrated feed medium started to be fed to the fermentor. The culture temperature and pH were maintained at 30 °C and 5.5, respectively. Cell growth was monitored by measuring the optical density at 600 nm. Glucose and galactose concentration were determined by using a chemical analyzer, YSI2700. Ethanol concentration was measured by using a gas chromatograph.

## Results and discussion

### Development of linguistic model for fed-batch culture

To investigate the performance of our algorithm, simulation studies for fed-batch culture with exponential feeding were carried out. A virtual model for fed-batch culture was used, which had been developed by the augmentation with the cell-inhibition term to the model for batch yeast culture. As shown in Fig. 1, the model obtained by previous stage showed similar trends in the low cell mass, but the discrepancy between the model-predicted and the process model grew rapidly with time. The model updater was executed successfully and the model could cope with the change of process characteristics well. It was found that the rule base and the membership function modified automatically as shown in Fig. 1.

### Development of optimization algorithm

Fed-batch culture for the production of yeast cells from glucose was selected as the model system and the objective was to maximize cellular productivity. To determine the optimum feed rate profile,  $F(t)$ , which maximizes the performance index, the culture time was divided into subintervals of fixed length and an optimum set of  $F_i$ 's was searched for. The performance of the developed genetic algorithm for four typical initial conditions were evaluated and compared with a previous approach<sup>2</sup> based singular control theory as in Table 1. The genetic algorithms showed better or similar performances compared with the singular control approach despite much less computational burden.

Table 1. Simulation results for different initial conditions

Initial conditions	Initial cell mass (g/L)	Initial glucose concentration (g/L)	Performance Index $IP(t_f)$	
			Genetic algorithms	Singular control (ref. (2))
IC I	0.2	0.0005	22.48	21.52
IC II	1.0	5.0	24.60	23.39
IC III	0.68	0.065	23.42	25.22
IC IV	1.0	0.0005	24.95	25.06

Initial volume : 2 L, Maximum allowable volume : 5 L,

Maximum feed flow rate : 1.0 L/hr, Total amount of glucose fed = 45 g,

Performance index (IP) =  $(XV)_f - 0.1t_f$

( $t_f$  : time when fermentor is full,  $(XV)_f$  : cell mass at  $t_f$ )

#### Real-time optimization of hPTH production

The model developed was used as the adaptive model. The concentrations of cells, glucose, galactose and ethanol were measured every one hour. When the difference between the model estimates of those concentrations and the measurement values exceeded a certain thresh-hold value, adaptation (model update) was carried out. With the updated model parameters, a new optimal feed rate profile for the remaining culture time was calculated by genetic algorithm. As shown in Figure 2, the model followed the actual process fairly well. The cell mass concentration reached 32 g/L at about 20 hour. The performance index was 382 mg and the maximum intact hPTH concentration was 178 mg/L at 20 hr, while the predicted value was about 197 mg/L. A summary of the results from several fed-batch cultures is given in Table 2. Real time optimization strategy achieved superior levels of performance when compared to galactose-only feeding and intermittent feeding of glucose and galactose. The discrepancy between the predicted values of protein concentration and the measured data was observed. The reasons were speculated that protein expression has complex and nonlinear nature and thus, low reproducibility and essentially it is impossible to analysis protein during operation and use feedback indicator.

Table 2. Effect of control strategies on fermentation performance

	intermittent feeding (galactose)	intermittent feeding (glucose and galactose)	Real time optimization
maximum hPTH (mg/L)	125	150	178
average volumetric productivity (mg/L · hr)	7.8	6.3	12.7

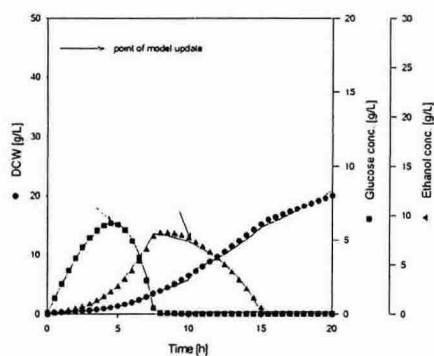


Fig. 1 State estimation of fed-batch culture using genetic-fuzzy system

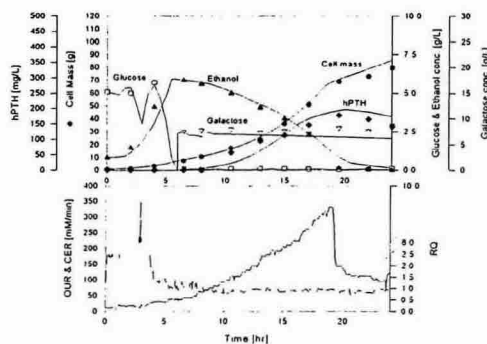


Fig. 2 Concentration profiles by real time optimization algorithm