ZENTRUM ANGEWANDTE CHEMIE

Institut für Technische Chemie

Institut für Technische Chemie, Callinstr. 3, 30167 Hannover

Partner in der Forschung

Leibniz Universität Hannover

Comparison of polysialic acid production during batch and fed-batch cultivations of *Escherichia Coli* K1

Ran Chen, Jinu John, Bernd Hitzmann and Thomas Scheper

Institut für Technische Chemie, Universität Hannover, Callinstr. 5, 30167 Hannover, Germany

Introduction

Polysialic acid (PSA) is a biocompatible and biodegradable polymer. It could provide the physico-chemical properties that cells could attach and proliferate, which make it very suitable for being used as scaffold material. As a promising biomaterial, PSA is needed in large amount thus this study is concentrated on improving the production of PSA by introducing fed-batch techniques. For the fed-batch cultivation of *Escherichia coli* K1 producing PSA, a controller has been developed. Based on the extended Kalman filter and supported by flow injection analysis (FIA) system, a feedback/feedforward controller has been established in order to maximize the PSA production and to minimize acetate production. Feeding control based on the on-line measurement of glucose concentration by the FIA system. The Kalman filter was used to reduce the noise of the glucose measurement by the FIA system and to estimate the biomass concentration, the glucose concentration, the maximal specific growth rate, and the culture broth volume Based on these values a controller was implemented to keep the glucose concentration to the desired set point of 0.05 g/L.

System setup

The enzyme, glucose oxidase (36µl,400U/ml) was injected into the carrier stream and then cell free samples(20µl) were injected.the amount of metabolized oxygenwas determined by an oxygen electrode as a measure for the glucose concertration.The control of the system were accomplished by CAFCA(computer Assited Flow Control and Analysis).

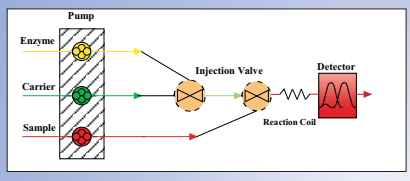

Fig. 2 Diagram of FIA system.

To minimize data noise the Kalman filter was applied (Hitzmann B,et al.Bioprocess Eng (2000) 23:337-341). The model was an ideal stirred tank reactor as well as the Monod equation for the limiting substrate glucose.The Kalman filter not only provided estimated data of glucose concentrations but also estimated data for biomass and the specific growth rate.The flow rate of the feeding pump was controlled based on these parameters and a PI controller.

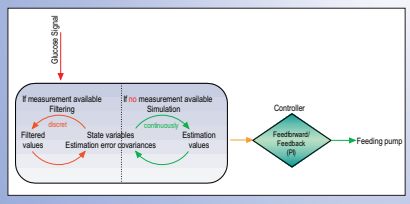

Fig. 3 Diagram of Kalman filter.

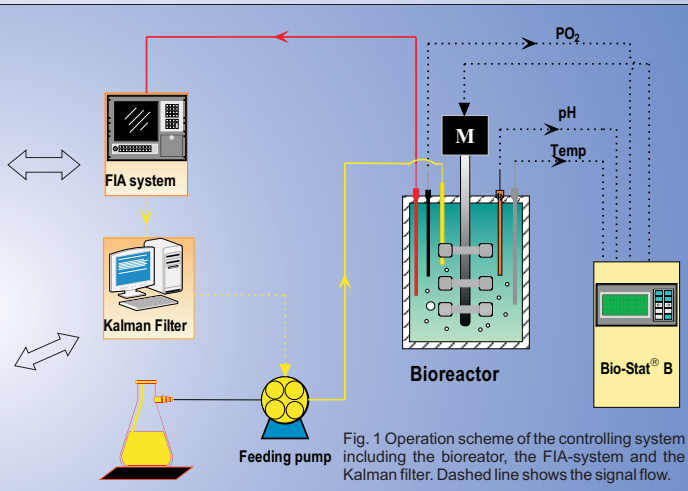

Fig. 1 Operation scheme of the controlling system including the bioreator, the FIA-system and the Kalman filter. Dashed line shows the signal flow.

Wild type strain *E.coli* B2032 serotype K1 was grown on a glucose mineral salt medium (Bastian R, et al .2008) in a 2 L bioreactor (Sartorius Stedim Biotech,Germany) with a working volume of 1.5 L in a fed-batch mode.The glucose control setpoint is 0.05 g/L.

Data of FIA and Kalman filter

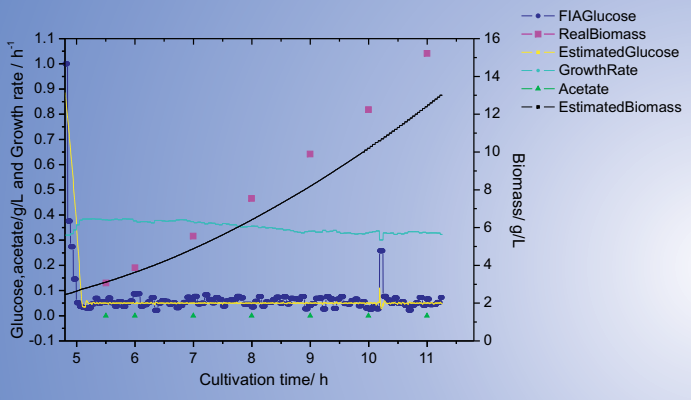

Fig. 4 Comprison of online glucose concentrations and estimated glucose concentrations; measured biomass and estimated biomass concentration; specific growth rate of the cells and acetate concentration in the medium were aslo shown. Feeding started at 4.8 h cultivation time.

Comparison of Batch and Fed-batch Cultivations

Table 1 PSA and acetate carbon yield and yield factors in relation to the applied amount of glucose in batch and fed-batch cultivations

Cultivation	Consumed Glucose (g)	PSA carbon yield $C_{PSA}/C_{glucose}$(%)	$Y_{PSA/glucose}$ (gg^{-1})	Acetate carbon yield $C_{acetate}/C_{glucose}$ (%)	$Y_{acetate/glucose}$ (gg^{-1})
Batch	31.42	3.07	0.0287	19.24	0.1922
Fed-Batch (8.8h)	31.42	4.38	0.0381	0	0
Fed-Batch (end)	54.93	4.68	0.0438	0	0

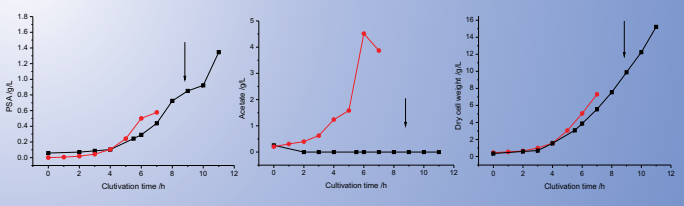

Fig. 5 Comparison of the process data of a 1.7 L batch (red dots) and a 1.5 L fed-batch (black square) cultivation. At the time point marked by arrows the glucose consuming in the fed-batch cultivation was equal to the total glucose applied in the batch cultivation.

Conclusions

Compared to the batch cultivation, PSA production increased from yield $_{PSA/Glucose}$ = 0.0287 $g_{PSA}/g_{Glucose}$ to 0.0438 $g_{PSA}/g_{Glucose}$ and acetate concentration decreased from 3.872 g/L to 0 which proved that fed-batch cultivation is more efficient for the PSA production.

Deutsche Forschungsgemeinschaft DFG

DFG Forschergruppe 548 Polysialinsäure
Evaluation eines neuen Werkstoffs als Gerüstsubstanz
polysia
für die Herstellung artifizieller Gewebe