Optimal Feed Rate Control of *Escherichia coli* Fed-batch Fermentation

Olympia Roeva*, Stoyan Tzonkov

Centre of Biomedical Engineering "Prof. Ivan Daskalov" - Bulgarian Academy of Sciences
105 Acad. G. Bonchev Str., 1113 Sofia, Bulgaria
E-mail: olympia@clbme.bas.bg, tzonkov@clbme.bas.bg
* Corresponding author

Received: December 10, 2004 **Accepted: March 31, 2005**

Published: April 28, 2005

***Abstract**: In this paper an optimal control algorithm for E. coli fed-batch fermentation has been developed. A simple material balance model is used to describe the E. coli fermentation process. The optimal feed rate control of a primary metabolite process is studied and a biomass production is used as an example. An optimization of a fed-batch fermentation process is usually done using the calculus of variations to determine an optimal feed rate profile. In the optimal control literature the problem is formulated as a free final time problem where the control objective is to maximise biomass at the end of the process. The obtained optimal feed rate profile consists of sequences of maximum and minimum feed rates. The obtained results are used for optimization of E. coli fed-batch fermentation and the presented simulations show a good efficiency of the developed optimal feed rate profile.*

***Keywords:** Optimal control, Fermentation process, Calculus of variations.*

Introduction

Cultivation of recombinant micro-organisms e.g. *Escherichia coli*, in many cases is the only economical way to produce pharmaceutical biochemicals such as interleukins, insulin, interferons, enzymes and growth factors. To maximize the volumetric productivities of bacterial cultures it is important to grow *E. coli* to high cell concentration. From different modes of operation, (batch, fed-batch and continuous), fed-batch operation is often used in industry due to its ability to overcome catabolite repression or glucose effect which usually occur during production of these fine chemicals. Moreover, fed-batch operation also gives the operator of freedom of manipulating the process via substrate feed rate. This sets the challenge to control and optimize fed-batch fermentation processes.

Optimization of fed-batch fermentation processes has been a topic of research for many years. Control opportunities in fed-batch operated fermentations have been reviewed in detail in a number of articles. It is well known that the design of high-performance modelbased control algorithms for biotechnological processes is hampered by two major problems which call for adequate engineering solutions. First, the process kinetics are too often poorly understood nonlinear functions, while the corresponding parameters are in general time-varying. Second, up to now there is a lack of reliable sensors suited to real-time monitoring of the process variables which are needed in advanced control algorithms. Therefore, the earliest attempts to control of a fermentation process did not use model at all.

High control algorithms, such as variable structure systems, etc., are elaborated to get over difficulties like measuring of the process variables and strongly time-varying parameters [2, 3, 7]. In this paper, an optimal control of the primary metabolite fermentation process, based on an optimal feed rate profile, is developed. The approaches used by many research groups to determine the substrate feed rate profile that optimises a desired objective function, are usually based on the calculus of variations [4, 5, 6, 8, 11, 12]. A biomass production is used as an example for the primary metabolite production process and the objective function is, therefore, to maximise biomass concentration at the end of the process. The mathematical representation of *E. coli* fed-batch fermentation process is developed in [1] and the optimal feed rate sequences that optimise this process is then formulated.

Description of the process

The cultivation of *E. coli MC4110* is performed in a 2l bioreactor (Bioengineering, Switzerland), using a mineral medium [1], in *Institut für Technische Chemie, Universität Hannover*. Before inoculation a glucose concentration of 2.5 g/l is established in the medium. Glucose in feeding solution is 100 g/l. Initial liquid volume is 1350 ml, pH is controlled at 6.8 and temperature is kept constant at 35^0C. The aeration rate is kept at 275 l/h air, stirrer speed at start 900 rpm, after 11h the stirrer speed is increased in steps of 100 rpm and at end is 1500 rpm. Oxygen is controlled around 35%.

The mathematical model of fed-batch fermentation of *E. coli* can be represented by the following dynamic mass balance equations:

$$\frac{dX}{dt} = \mu_{max} \frac{S}{K_S + S} X - \frac{F}{V} X \tag{1}$$

$$\frac{dS}{dt} = -\frac{1}{Y_{XS}} \mu_{max} \frac{S}{K_S + S} X + \frac{F}{V}\left(S_{in} - S\right) \tag{2}$$

$$\frac{dV}{dt} = F \tag{3}$$

where: X is the concentration of biomass, [g/l];
S - concentration of substrate (glucose), [g/l];
F - feeding rate, [l/h];
V - bioreactor volume, [l];
S_{in} - concentration of the feeding solution, [g/l];
μ_{max} - maximum growth rate, [h^{-1}];
K_s - the substrate concentration at which half the maximum specific growth rate is obtained (μ=0.5μ_{max}), [g/l];
Y_{XS} – the yield of cell mass from substrate, [g cell/g substrate].

Development of an optimal feed rate control

Problem statement

The fed-batch fermentation is constrained by the restrictions of permissible final volume V_f at the time of the end of the process t_f, and minimum and maximum of substrate feed rates:

$$0 \le F \le F_{\max} \tag{4}$$

$$V(t_f) = V_f \tag{5}$$

The aim of this primary metabolite (biomass) production is to maximise the biomass concentration (*X*) at the end of the fermentation process using substrate feed rate (*F*). This task can be transformed into an objective function as [12]:

$$J(F) = X(t_f) + \varepsilon \int_{t_0}^{t} dt \tag{6}$$

Here ε is the cost factor per unit of operating time. In case of non-monotonic growth kinetics, it is noticed [11] that without the presence of cost factor, the necessary condition for the singular period can not be specified. However the considered growth kinetics in the model of *E. coli* (1) – (3) does not require the existence of singular feed rate [9], the cost factor is added to objective function *J* (6) for a completeness.

The state optimization problem can be solved using the calculus of variation [12]. Due to the specific structure of the model (1) – (3), a Hamiltonian equation which is affine in the control input is obtained. Therefore, for this process the Hamiltonian equation can be written as:

$$\begin{aligned} H = -\varepsilon + \lambda_X \left(\mu_{max} \frac{S}{K_S + S} X - \frac{F}{V} X \right) + \\ + \lambda_S \left(-\frac{1}{Y_{XS}} \mu_{max} \frac{S}{K_S + S} X + \frac{F}{V}(S_{in} - S) \right) + \\ + \lambda_V F \end{aligned} \tag{7}$$

and the costate equations:

$$\dot{\lambda}_X = -\frac{\partial H}{\partial X} = -\lambda_X \left(\mu_{max} \frac{S}{K_S + S} - \frac{F}{V} \right) + \lambda_S \frac{1}{Y_{XS}} \mu_{max} \frac{S}{K_S + S} \tag{8}$$

$$\dot{\lambda}_S = -\frac{\partial H}{\partial S} = -\lambda_X \frac{\mu_{max} K_S X}{(K_S + S)^2} + \lambda_S \left(\frac{1}{Y_{XS}} \frac{\mu_{max} K_S X}{(K_S + S)^2} + \frac{F}{V} \right) \tag{9}$$

$$\dot{\lambda}_V = -\frac{\partial H}{\partial V} = -\lambda_X \frac{XF}{V^2} + \lambda_S \frac{F}{V^2}(S_{in} - S) \tag{10}$$

The transversality or final conditions can also be written as:

$$\lambda_X(t_f) = \frac{\partial J}{\partial X_{t_f}} = 1 \text{ and } \lambda_S(t_f) = 0 \tag{11}$$

The optimal feed rate sequences are then calculated from Equation (12) in which the sign of Ψ is used to indicate the period of maximum, minimum or singular feed rate. As a result, the optimal control is of the bang-bang type, with the possibility of singular arcs depending on the value of Ψ.

$$\frac{\partial H}{\partial F} = -\lambda_X \frac{X}{V} + \lambda_S \frac{(S_{in} - S)}{V} + \lambda_V = \Psi \qquad (12)$$

if $\Psi < 0$ then $F = 0$
if $\Psi > 0$ then $F = F_{max}$
if $\Psi = 0$ then $F = F_{sing}$

The singular feed rate (F_{sing}) can be determined by differentiating equation (12) until feed rate (F) reappears in the equation. In the case of considered Monod growth kinetics $\Psi \neq 0$ [9], therefore there is no F_{sing}.

Simulation results

The simulations of the process model (1) – (3) with the developed optimal feed rate profile are done. The used values of the model parameters are presented in Table 1.

Table 1. Numerical values of stoichiometric and kinetic coefficients in the model

Coefficient	$\mu_{\max}$	K_s	Y_{XS}
Value	0.59 h^{-1}	0.045 g/l	2.00 gg^{-1}

The initial conditions of the process variables and the concentration of the feeding solution are:

$X(0)$ = 1.252 g/l, $S(0)$ = 0.812 g/l,
$V(0)$ = 1.350 l and S_{in} = 100 g/l.

Three optimal feed rate profiles are calculated, respectively for F_{max} = 0.13 l/h, F_{max} = 0.17 l/h and F_{max} = 0.23 l/h. The results for biomass concentration at the end of the process - $X(t_f)$, are listed in Table 2.

Table 2. Numerical values of the concentration of biomass at the end of the process

Feed rates	$X(t_f)$
original feed rate	8.2074 g/l
F_{max} = 0.13 l/h	10.0763 g/l
F_{max} = 0.17 l/h	12.9603 g/l
F_{max} = 0.23 l/h	14.9613 g/l

The biomass concentrations received with the different values for F_{max} compared with original experimental data are presented in Fig. 1. Fig. 2 demonstrates the original feed rate. Fig. 3 shows the optimal feed rate profile in case of F_{max}=0.23 l/h, because two other optimal profiles at F_{max} = 0.13 l/h and F_{max} = 0.17 l/h are similar.

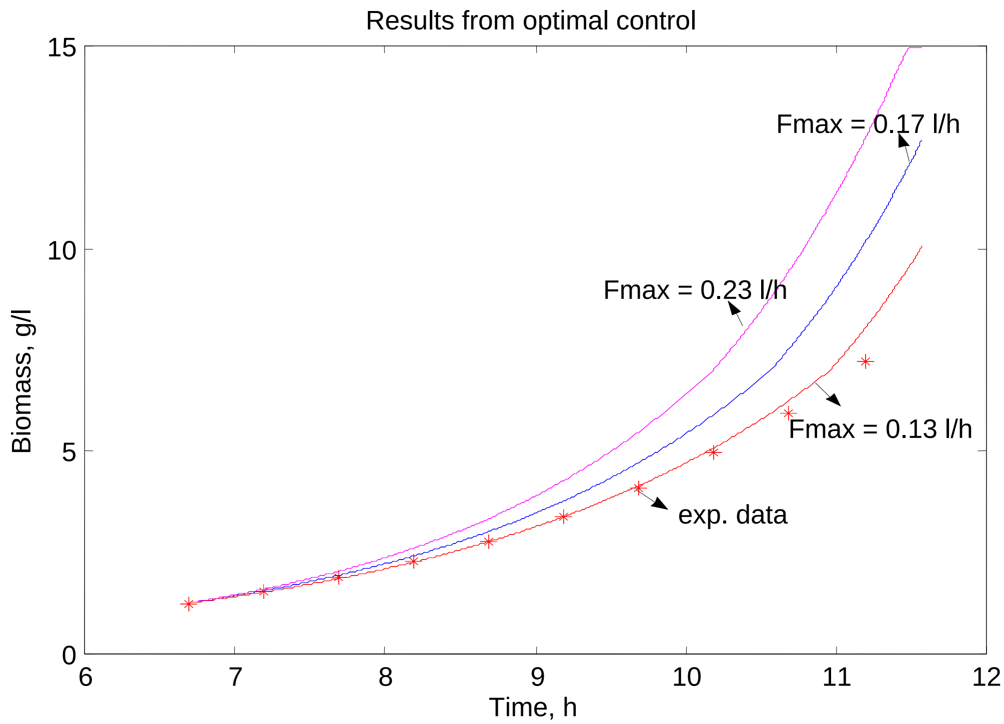
Results from optimal control
Biomass, g/l
Time, h
Fmax = 0.17 l/h
Fmax = 0.23 l/h
Fmax = 0.13 l/h
exp. data
0
5
10
15
6
7
8
9
10
11
12

Fig. 1

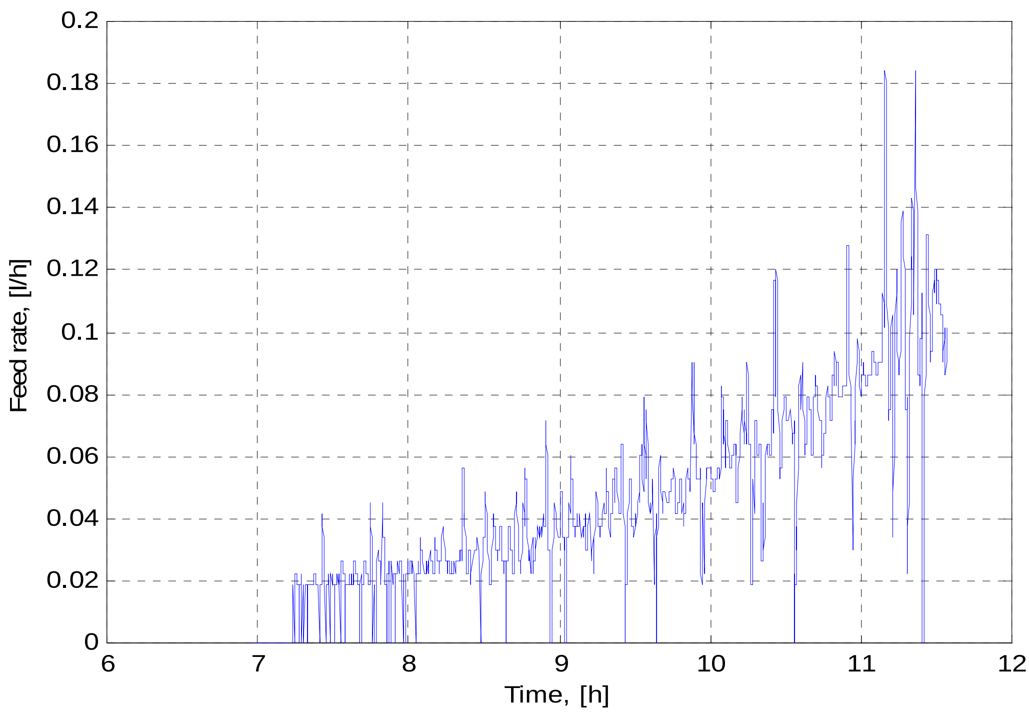
Feed rate, [l/h]
Time, [h]
0
0.02
0.04
0.06
0.08
0.1
0.12
0.14
0.16
0.18
0.2
6
7
8
9
10
11
12

Fig. 2

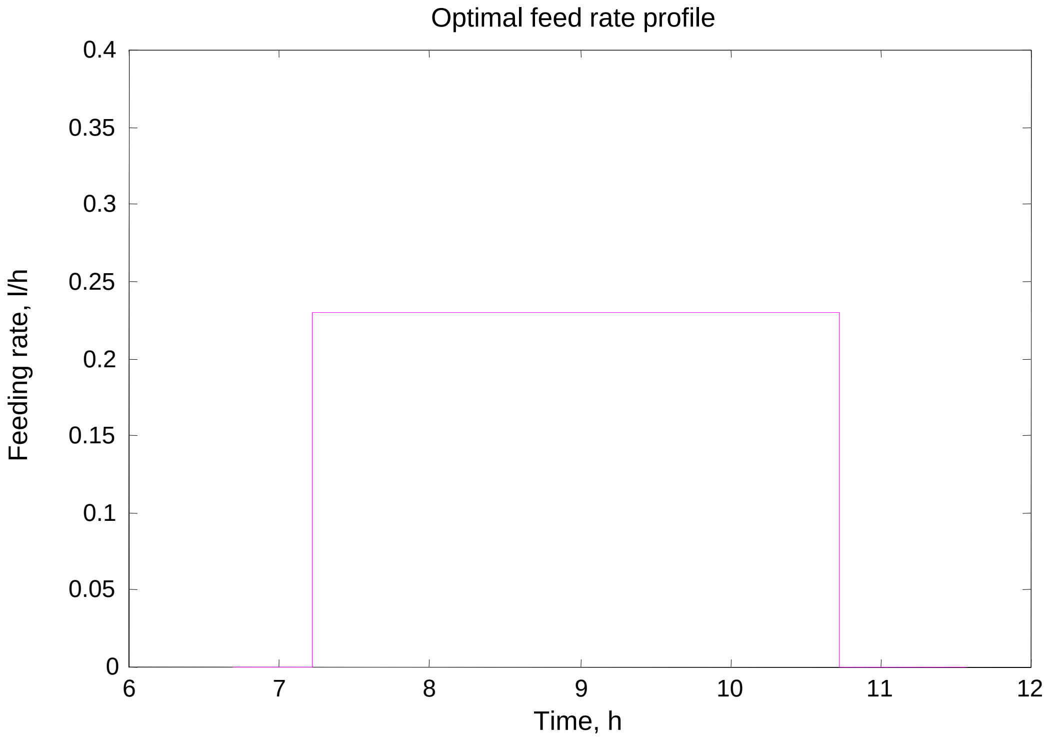

Fig. 3

Presented results show that the developed optimal feed rate profiles lead to the increasing of biomass concentration at the end of the considered process with 18.55% in case of F_{max}=0.13 l/h, with 36.67% in case of F_{max}=0.17 l/h and with 45.17 % in case of F_{max}=0.23 l/h.

Conclusion

In this paper some optimal feed rate profiles for *E. coli* fed-batch fermentation have been developed. A simple material balance model is used to describe the *E. coli* fermentation process. An optimization of the presented fed-batch fermentation process is done using the calculus of variations. Control objective is to maximise biomass concentration at the end of the process. The obtained optimal feed rate profiles consists of sequences of maximum and minimum feed rates. The presented results and simulations show a good efficiency of the developed optimal feed rate profiles. The synthesized optimal control provides increasing of biomass concentration at the end of the considered process up to 45.17 %.

However, an important drawback of the optimal control solution is that the optimal control is a very model sensitive technique. It requires a complete knowledge of the process model, including an analytic expression for all specific kinetics rates. Since in biotechnology this assumption is never fulfilled in practice, the optimal profile is generally calculated using a highly simplified model describing the process more or less correctly only from a qualitative view-point. Therefore, for future research, it is very useful to construct *suboptimal* strategies that do not suffer from the above difficulties.

Acknowledgements

This work is partially supported from FNSF-TS-1314/2003 and DFG 113/135.

References

1. Georgieva O., M. Arndt, B. Hitzmann (2001). Modelling of *Escherichia coli* Fed-batch Fermentation, International Symposium "Bioprocess Systems'2001 -BioPS'2001", 1-3.X, Sofia, Bulgaria, I.61-I.64.
2. Hisbullah, M. A. Hussain, K. B. Ramachandran (2002) Comparative Evaluation of Various Control Schemes for Fed-batch Fermentation, Bioprocess and Biosystems Engineering, 24, 309-318.
3. Hristozov I., T. Pencheva, B. Hitzmann (2001). Implementation of Variable Structure Control Based on Flow Injection Measurements for Fed-Batch Cultivation of Saccharomyces cerevisiae, International Symposium "Bioprocess Systems'2001 - BioPS'2001", 1-3.X, Sofia, Bulgaria, I.53-I.56.
4. Jadot F., G. Bastin, J. F. Van Impe (1998). Optimal Adaptive Control of a Bioprocess with Yield-productivity Conflict, Journal of Biotechnology, 65, 61-68.
5. Pavlov, Y., K. Ljakova (2004). Equivalent Models and Exact Linearization by the Optimal Control of Monod Kinetics Models, Bioautomation, 1, 42-56**.**
6. Rocha I., E. C. Ferreira (2002). Optimization Methods for Improving Fed-batch Cultivation of *E. coli* Producing recombinant proteins, Proc. of the 10^{th} Mediterranean Conference on Control and Automation – MED2002, Lisabon, Portugal, July 9-12.
7. Smets I. Y., G. P. Bastin, J. F. Van Impe (2002). Feedback Stabilization of Fed-Batch Bioreactors: Non-Monotonic Growth Kinetics, Biotechnol. Prog. 18, 1116-1125.
8. Smets I. Y., J. E. Claes, E. J. November, G. P. Bastin, J. F. Van Impe (2003). Optimal Adaptive Control of (Bio)chemical Reactors: Past, Present and Future, Journal of Process Control (Special Issue), November, 1-43.
9. Staniskis J., D. Levisauskas, R. Simutis, U. Viestur, M Kristapsons (1992). Automation of Biotechnological Processes. Measurements, Optimization and Control, Zinatne, Riga.
10. Staniskis J. (1984). Optimal Control of Biotechnical Processes, Mokslas, Vilnius.
11. Van Impe J.F., G. Bastin (1995). Optimal Adaptive Control of Fed-Batch Fermentation Processes, Control Eng. Practice, 3 (7), 939-954.
12. Vanichsriratana W. (2000). Optimization of a Primary Metabolite Fermentation Process: Effect of Cost Factor on the Optimal Feed Rate Control, Kasetsart J. (Nat. Sci.) 34, 495-499.