Optimal Feeding Trajectories Design for *E. coli* Fed-batch Fermentations

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Abstract: *In this paper optimal control algorithms for two E. coli fed-batch fermentations are developed. Fed-batch fermentation processes of E. coli strain MC4110 and E. coli strain BL21(DE3)pPhyt109 are considered. Simple material balance models are used to describe the E. coli fermentation processes. The optimal feed rate control of a primary metabolite process is studied and a biomass production is used as an example. The optimization of the considered fed-batch fermentation processes is done using the calculus of variations to determine the optimal feed rate profiles. The problem is formulated as a free final time problem where the control objective is to maximize biomass at the end of the process. The obtained optimal feed rate profiles consist of sequences of maximum and minimum feed rates. The resulting profiles are used for optimization of the E. coli fed-batch fermentations. Presented simulations show a good efficiency of the developed optimal feed rate profiles.*

Keywords: *Optimal control, Calculus of variations, E. coli fed-batch fermentation.*

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. This sets the challenge to control and optimize fed-batch fermentation processes.

In recent years, the problem of determining optimal controls for fed-batch fermentation processes has become an important field of interest in biochemical engineering. 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. In these cultivation processes, the substrate is fed into the bioreactor intermittently or continuously during the whole process. For many biochemical processes, this technique improves the product output upon the so-called batch-fermentation technique, where the whole substrate is given into the bioreactor a priori. The problem is to determine optimal feed rate profiles, such that the final product is maximized. The approaches used by many research groups to determine the substrate feed rate profile that optimizes a desired objective function, are usually based on the calculus of variations [3, 7, 9, 11, 13, 14].

The calculus of variations is a mathematical procedure that exists independently of Hamilton's Principle. The method is connected with the conctruction of optimal shaped, states, or processes where the optimality criterion is given in the form of an integral involving an unknown function. The task of the calculus of variations then is to demonstrate the existence and to deduce the properties of some function that realizes the optimal value of this integral. Such variational problems accur in many-fold applications, in particular in physics, engineering and economics, and the variational integral may represent some action, energy, or cost functional [5].

In this paper, classical solutions to minimization/maximization problems in the calculus of variations are prescribed by an optimal control of the primary metabolite fermentation process, based on optimal feed rate profiles. Two fed-batch fermentation models of the *E. coli* strain *MC4110* and strain *BL21(DE3)pPhyt109* are considered [2, 10]. A biomass production is used as an example for the primary metabolite production process. The objective function is to maximize biomass concentration at the end of the process. The optimal feed rate sequences that optimize these processes are formulated.

E. coli fermentation processes

Fed-batch fermentation processes of *E. coli* strain *MC4110* and strain *BL21(DE3)pPhyt109* were studied [1, 5, 6, 8]. A brief description of the fermentation conditions is presented below.

Fed-batch fermentation of E. coli MC4110

The fermentation is performed in a 2 l bioreactor (Bioengineering, Switzerland), using a mineral medium, in *Institute of Technical Chemistry, University of Hannover*. Before inoculation a glucose concentration of 2.5 $g \cdot l^{-1}$ is established in the medium. Glucose in feeding solution is 100 $g \cdot l^{-1}$. Initial liquid volume is 1350 ml, pH is controlled at 6.8 and temperature is kept constant at 35°C. The aeration rate is kept at 275 $l \cdot h^{-1}$ air, stirrer speed at start 900 rpm, after 11 h the stirrer speed is increased in steps of 100 rpm and until reaching is 1500 rpm. Oxygen is controlled around 35%.

For off-line glucose measurements as well as biomass and acetate concentration determination samples of about 10 ml are taken roughly every hour. Off-line measurements are performed by using the Yellow Springs Analyzer (Yellow Springs Instruments, USA). For on-line glucose determination a flow injection analysis (FIA) system has been employed using two pumps for a continuous sample and carrier flow rate.

Fed-batch fermentation of E. coli BL21(DE3)

E. coli strain *BL21(DE3)pPhyt109* is used for fermentation experiments. The experiments are performed in the *Department of Fermentation Engineering, Faculty of Technology, University of Bielefeld*. Plasmid pPhyt109, an expression vector derived from the multi copy plasmid pUC19, contains the gene for *E. coli* phytase under the constitutive promoter of the *bglA* gene of *Bacillus amyloliquefaciens*.

Fermentation experiments are carried out in a bioreactor with a total volume of 7 l and a working volume of 5 l. The bioreactor is equipped with direct digital control (DDC) from MBR (*Multiple Bioreactors and Sterile Plants*, *Zurich*, *Switzerland*). Glucose mineral salt medium is used as growth medium. The pH is maintained at 6.9 by controlled addition of 4 N NaOH. Antifoam (*PE8100*, *BASF*, *Germany*) is added automatically when required. The

temperature is kept at 37°C. Air flow is kept constant at 10 l·min^{-1}. The stirrer speed is kept constant at 500 rpm.

Samples were obtained for measurements of biomass, glucose, acetate and phytase using the automatic sampler FC205 (*Gilson*, *Middleton*, *USA*) and stored at 4°C until analysed. 1 ml of culture broth was centrifuged in *Eppendorf* vials at 14 000 rpm for 10 min; the pellet was washed twice in an aqueous NaCl solution and dried overnight at 60°C under vacuum before bacterial dry mass was determined. Using test kits, glucose and acetate were determined. The phytase activity and carbon dioxide was measured too.

Optimal feeding trajectories design

The fed-batch fermentation is constrained by the restrictions of permissible final volume, and minimum and maximum of substrate feed rates:

$$0 \le F \le F_{\max} \tag{1}$$

$$V(t_f) = V_f \tag{2}$$

The aim of this primary metabolite (biomass) production is to maximize 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 [14]:

$$J(F) = X(t_f) + \varepsilon \int_{t_0}^{t} dt \tag{3}$$

Here ε is the cost factor per unit of operating time. In case of non-monotonic growth kinetics, it is noticed [13] 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 models of *E. coli* (4)-(6) and (13)-(16) does not require the existence of singular feed rate [12], the cost factor is added to objective function J (Eq. (3)) for completeness.

Feeding trajectory design of E. coli MC4110

The following assumptions are made for the models development of the considered fed-batch fermentations of *E. coli*:

- The bioreactor is completely mixed.
- Potential mixing effects of the highly concentrated feeds with the fermentation medium are neglected for the sake of the model simplicity.
- The suspension viscosity in the reactor remains constant during the experiment.
- The substrate (glucose) is consumed mainly oxidatively.
- Variations in the growth rate, as well as in substrate consumption do not significantly change the elemental composition of biomass, thus balanced growth conditions are only assumed.
- Parameters, e.g. pH and temperature, are controlled to certain acceptable constant values during the process.

The mathematical model of fed-batch fermentation of *E. coli MC4110* can be represented by the following dynamic mass balance equations:

$$\frac{dX}{dt} = \mu_{\max} \frac{S}{k_S + S} X - \frac{F}{V} X \tag{4}$$

$$\frac{dS}{dt} = -\frac{1}{Y_{XS}}\mu_{\max}\frac{S}{k_S + S}X + \frac{F}{V}(S_{in} - S) \tag{5}$$

$$\frac{dV}{dt} = F \tag{6}$$

where: X is the concentration of biomass, [g·l^{-1}]; S – concentration of substrate, [g·l^{-1}]; F – feeding rate, [l·h^{-1}]; V – bioreactor volume, [l]; S_{in} – substrate concentration of the feeding solution, [g·l^{-1}]; $\mu_{\max}$ – maximum growth rate, [h^{-1}]; k_S – saturation constant, [g·l^{-1}]; Y_{XS} – yield coefficient, [g·g^{-1}].

The state optimization problem can be solved using the calculus of variation [14]. Due to the specific structure of the model (4) – (6), 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 Eq. (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.

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

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

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

Simulation results

The simulations of the process model (4)-(6) with the developed optimal feed rate profile are done. The used values of the model parameters are presented in Table 1 [2].

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

Coefficient	μ_{max}	k_S	Y_{XS}
Value	0.59 h^{-1}	0.045 $g \cdot l^{-1}$	2.00 $g \cdot g^{-1}$

The initial conditions of the process variables and the concentration of the feeding solution are [2]:

$$X(0) = 1.252 \text{ g} \cdot \text{l}^{-1}, \quad S(0) = 0.812 \text{ g} \cdot \text{l}^{-1}, \quad V(0) = 1.350 \text{ l}, \quad S_{in} = 100 \text{ g} \cdot \text{l}^{-1}.$$

Three optimal feed rate profiles are calculated, respectively for $F_{max} = 0.13 \text{ l} \cdot \text{h}^{-1}$, $F_{max} = 0.17 \text{ l} \cdot \text{h}^{-1}$and $F_{max} = 0.23 \text{ l} \cdot \text{h}^{-1}$. The values of F_{max} are chosen accordingly process particularities [2]. 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 $g \cdot l^{-1}$
$F_{max} = 0.13 \text{ l} \cdot \text{h}^{-1}$	10.0763 $g \cdot l^{-1}$
$F_{max} = 0.17 \text{ l} \cdot \text{h}^{-1}$	12.9603 $g \cdot l^{-1}$
$F_{max} = 0.23 \text{ l} \cdot \text{h}^{-1}$	14.9613 $g \cdot l^{-1}$

The biomass concentrations received with the different values for F_{max} compared with original experimental data are presented in Fig. 1. The optimal feed rate profile in case of $F_{max} = 0.23 \text{ l} \cdot \text{h}^{-1}$ is presented in Fig. 2.

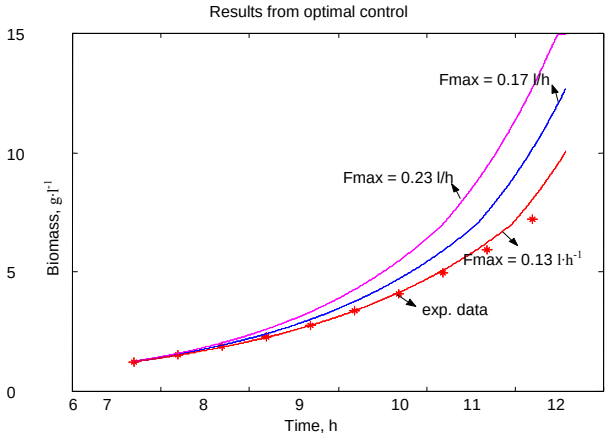

Fig. 1 Obtained biomass and phytase concentrations compared to the original experimental data

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^{-1}, with 36.67% in case of F_{max} = 0.17 l·h^{-1}and with 45.17% in case of F_{max} = 0.23 l·h^{-1}.

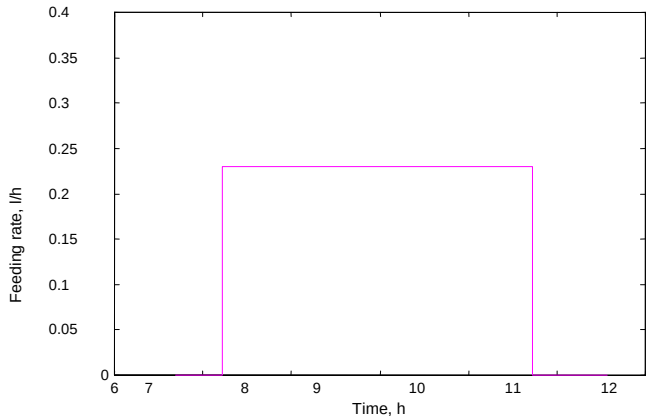

Fig. 2 Optimal feed rate profile in case of F_{max} = 0.23 l·h^{-1}

Feeding trajectory design of E. coli BL21(DE3)pPhyt109

The rates of cell growth, substrate consumption and phytase production are commonly described according to the mass balance as follows:

$$\frac{dX}{dt} = \mu_{max} \frac{S}{k_S + S} X - \frac{F}{V} X \tag{13}$$

$$\frac{dS}{dt} = -\frac{1}{Y_{XS}} \mu_{max} \frac{S}{k_S + S} X + \frac{F}{V}(S_{in} - S) \tag{14}$$

$$\frac{dPh}{dt} = \frac{1}{Y_{XPh}} \mu_{max} \frac{S}{k_S + S} X - \frac{F}{V} Ph \tag{15}$$

$$\frac{dV}{dt} = F \tag{16}$$

where: X is the concentration of biomass, [g·l^{-1}]; S – concentration of glucose, [g·l^{-1}]; Ph – concentration of phytase, [g·l^{-1}]; F – feeding rate, [l·h^{-1}]; V – bioreactor volume, [l]; S_{in} – substrate concentration of the feeding solution, [g·l^{-1}]; μ_{max} – maximum growth rate, [h^{-1}]; k_S – saturation constant, [g·l^{-1}]; Y_{XS}, Y_{XPh} – yield coefficients, [g·g^{-1}].

Analogically to the previous case, the optimal feed rate sequences are calculated.

Simulation results

The used values of the model parameters are presented in Table 3. The initial conditions of the process variables and the concentration of the feeding solution are [1]:

$X(0)$ = 2.38 g·l^{-1}, $S(0)$ = 0.84 g·l^{-1}, $P(0)$ = 5.84 g·l^{-1}, $V(0)$ = 2.70 l, S_{in} = 500 g·l^{-1}.

Table 3. Numerical values of stoichiometric and kinetic coefficients in the model

Coefficient μ_{max}	k_S	Y_{XS}	Y_{XP}
Value 0.58 h^{-1}	0.05 $g \cdot l^{-1}$	2.26 gg^{-1}	3.50 gg^{-1}

Four optimal feed rate profiles are calculated, respectively for: F_{max} = 0.07 $l \cdot h^{-1}$, F_{max} = 0.10 $l \cdot h^{-1}$, F_{max} = 0.12 $l \cdot h^{-1}$and F_{max} = 0.15 $l \cdot h^{-1}$. The values of F_{max} are chosen accordingly process particularities [1]. The results for the biomass and phytase concentrations at the end of the process ($X(t_f)$, $P(t_f)$), are listed in Table 4.

The biomass and phytase concentrations received with the different F_{max}, compared to the original experimental data, are presented in Fig. 3 and Fig. 4. The optimal feed rate profile in case of F_{max} = 0.10 $l \cdot h^{-1}$ is presented in Fig. 5.

Table 4. Numerical values of the biomass and phytase concentrations at the end of the process

Feed rates	$X(t_f)$	$P(t_f)$
original feed rate	34.50 $g \cdot l^{-1}$	127.48 $g \cdot l^{-1}$
F_{max} = 0.07 $l \cdot h^{-1}$	31.02 $g \cdot l^{-1}$	96.03 $g \cdot l^{-1}$
F_{max} = 0.10 $l \cdot h^{-1}$	47.47 $g \cdot l^{-1}$	140.38 $g \cdot l^{-1}$
F_{max} = 0.12 $l \cdot h^{-1}$	58.37 $g \cdot l^{-1}$	166.97 $g \cdot l^{-1}$
F_{max} = 0.15 $l \cdot h^{-1}$	74.21 $g \cdot l^{-1}$	201.87 $g \cdot l^{-1}$

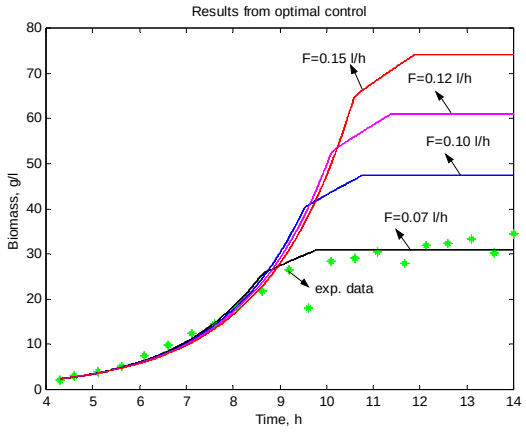

Fig. 3 Obtained biomass concentrations compared to the original experimental data

Presented results show that the developed optimal feed rate profiles lead to the increasing of the biomass concentration at the end of the process by:

27.32% in the case of F_{max} = 0.10 $l \cdot h^{-1}$,
40.89% in the case of F_{max} = 0.12 $l \cdot h^{-1}$
and
53.51% in the case of F_{max} = 0.15 $l \cdot h^{-1}$.

Respectively, the phytase concentration, at the end of the process, is increased by:

9.20% in the case of F_{max} = 0.10 $l \cdot h^{-1}$,
23.65% in the case of F_{max} = 0.12 $l \cdot h^{-1}$
and
36.85% in the case of F_{max} = 0.15 $l \cdot h^{-1}$.

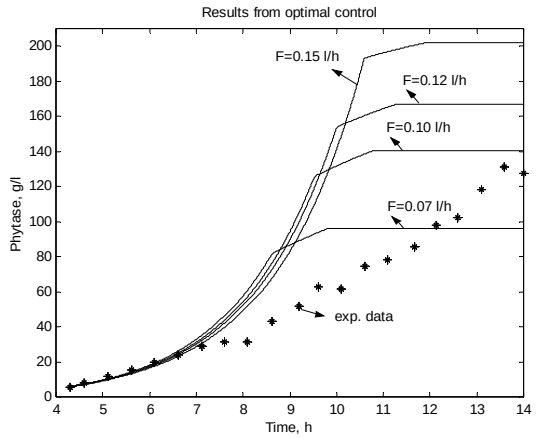

Fig. 4 Obtained phytase concentrations compared to the original experimental data

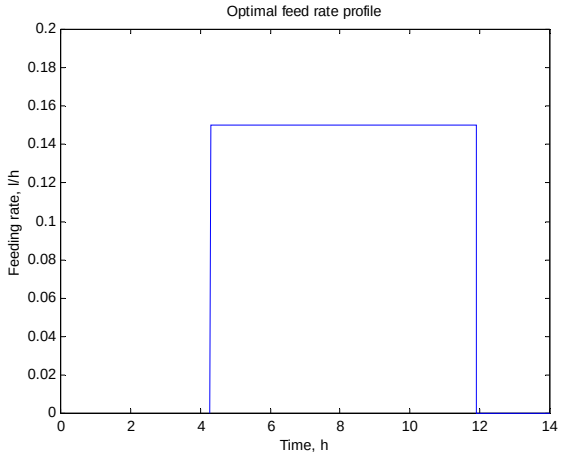

Fig. 5 Optimal feed rate profile in case of F_{max} = 0.15 l·h^{-1}

Conclusions

In this paper a set of optimal feed rate profiles for two strain *E. coli* fed-batch fermentation was developed. Two systems of differential equations were used for mathematical description of the *E. coli MC4110* and *E. coli BL21(DE3)pPhyt109* fed-batch fermentation processes. Optimization of the considered processes was done using the calculus of variations. The control objective was to maximize the biomass concentrations at the end of the process. The obtained optimal feed rate profiles were consisted of sequences of maximum and minimum feed rates. The presented results and simulations show a good efficiency of the developed optimal feed rate profiles. For *E. coli MC4110* fermentation the synthesized optimal control provides increasing of biomass concentration at the end of the process up to 45.17%. For *E. coli BL21(DE3)pPhyt109* fermentation in the case of F_{max} = 0.15 l·h^{-1} the synthesized optimal control provides increase of the biomass concentration (up to 53%) and phytase concentration (up to 36%) at the end of the process.

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.

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