Parameter Estimation of a Model Describing the Oxygen Dynamics in a fed-batch E. coli Cultivation

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PARAMETER ESTIMATION OF A MODEL DESCRIBING THE OXYGEN DYNAMICS IN A FED-BATCH *E. COLI* CULTIVATION

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Abstract A model describing the oxygen dynamics in an *E. coli* fed-batch cultivation is presented. In a linearised version the parameters are estimated and validated with good results. The model is used to discuss the guidelines for feed controller tuning derived in (Åkesson *et al.*, 2001b).

Key words Bio-reactor model, parameter estimation, *E. coli* fermentation.

1. INTRODUCTION

Today many proteins are produced by genetically modified microorganisms. One of the host organisms used is the bacterium *Escherichia coli*. To achieve a good productivity, high cell concentration and high cell productivity are desired and this is usually obtained through fed-batch cultivations. Much work is done on how to determine the addition of the growth-limiting carbon, often glucose, (Riesenberg and Guthke, 1999), (Lee, 1996). This is important as underfeeding will lead to productivity loss and starvation. Overfeeding leads to carbon nutrient accumulation or by-product formation, such as acetate. Acetate production reduces growth and recombinant protein production, (Luli and Strohl, 1990). In (Åkesson *et al.*, 2001a) a probing feeding strategy is presented. By superimposing short pulses on the substrate feed, on-line detection of acetate formation is made using the dissolved oxygen sensor. A feedback algorithm is used to adjust the feed rate to avoid overflow metabolism and thereby acetate formation while maintaining a high growth. To derive guidelines on the tuning of the feed controller, a linearised model is used in (Åkesson *et al.*, 2001b). Here the model is extended and verified. As the model is based on physical principles it is a continuous-time system. Reviews on identification and parameter estimation in continuous time are given in (Unbehauen and Rao, 1998), (Unbehauen and Rao, 1990) and (Young, 1981). The model is not used for on-line control and therefore the parameter estimation is done off-line. Also, the effect of the extended model on the tuning rules is investigated.

2. PROCESS DESCRIPTION

The process is a bio-reactor operating in fed-batch mode. Here we consider the case with two inputs: the stirrer speed N and the feed rate F, and three on-line outputs: the oxygen concentration in the airflow O_2 which is measured using a gas analyser, the dissolved oxygen concentration in the medium DO and the reactor medium volume V. The cell mass is measured off-line, see figure 1. A full non-linear model of the bio-reactor is presented together with a linear version. In this model also the changing oxygen concentration in the outlet air is included, equation (6), which is not the case in (Åkesson *et al.*, 2001b).

2.1 *Full model*

Mass balances of a fed-batch bio-reactor are

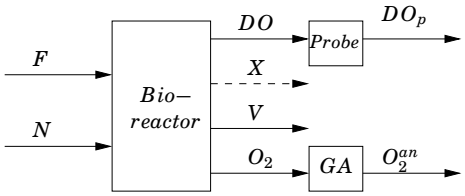

Fig. 1 Block diagram of the process. DO dissolved oxygen concentration, F feed rate, N stirrer speed, V the reactor medium volume, X cell mass concentration, GA = gas analyser, probe = dissolved oxygen probe.

given by:

$$\frac{dV}{dt} = F \tag{1}$$

$$\frac{d(VG)}{dt} = FG_{in} - q_g(G)VX \tag{2}$$

$$\frac{d(VA)}{dt} = q_a(G,A)VX \tag{3}$$

$$\frac{d(VX)}{dt} = \mu(G)VX \tag{4}$$

$$\frac{d(VC_o)}{dt} = K_La(N)V(C_o^* - C_o) - q_o(G)VX \tag{5}$$

$$\frac{d(V_gO_2)}{dt} = Q(O_2^{in} - O_2) - \frac{RTK_La(N)V}{PM}(C_o^* - C_o) \tag{6}$$

The expressions for the growth rate μ, the acetate consumption q_a, the oxygen consumption q_o and the glucose uptake q_g are given in the appendix. For notation and parameter values, see table 1 and table 3. The gas volume in the reactor V_g is given by

$$V_g = V_{tot} - V$$

where V_{tot} is the reactor volume. Henry's law gives the dissolved oxygen concentration DO in %:

$$DO = HC_o$$

Oxygen concentration in the outlet air O_2 is related to oxygen concentration in equilibrium with the gas bubbles in the reactor, C_o^* and DO^*, as

$$O_2 = \frac{HC_o^*O_2^{in}}{100} = \frac{DO^*O_2^{in}}{100} \tag{7}$$

This is based on the assumption that the gas bubbles are well mixed in a small stirred reactor (Enfors and Häggström, 1994). The dissolved oxygen sensor dynamics is approximated as:

$$T_p\frac{dDO_p}{dt} + DO_p = DO \tag{8}$$

together with a time delay denoted L_p. The gas analyser is described by:

$$T_{an}\frac{dO_2^{an}}{dt} + O_2^{an} = O_2 \tag{9}$$

together with a time delay denoted L_{an}.

Table 1 Variables in the model.

Symbol	Description
V	reactor medium volume
O_2	oxygen conc in outlet air
F	glucose feed into the reactor
N	stirrer speed
G	glucose concentration
A	acetic acid concentration
X	cell mass concentration
$C_o^{(*)}$	(sat.) dissolved oxygen conc.

2.2 *Linearised model*

Linearised versions of equations (2), (5) and (6) with respect to F, N, q_g, DO and DO^* when $q_o < q_o^{max}$ and no acetic acid is present are presented. Also the relations in equation (7) and equation (11) in appendix are used. The influences from the deviations $\Delta X = X - X^o$ and $\Delta V = V - V^o$ are assumed to be small and are therefore neglected.

$$T_g\frac{d\Delta q_g}{dt} + \Delta q_g = K_{gf}\Delta F$$

$$T_o\frac{d\Delta DO}{dt} + \Delta DO = K_{og}\Delta q_g + K_N\Delta N + \Delta DO^*$$

$$T_o^*\frac{d\Delta DO^*}{dt} + \Delta DO^* = K_{o^*o}\Delta DO + K_{o^*N}\Delta N$$

The linearised parameters are given in the appendix. After the introduction of $p = \frac{100RTV^o}{O_2^{in}HMPV_g^o}$, $K_{gf}K_{og}^{new} = \frac{K_{gf}K_{og}}{T_o}$ and the dynamics of the sensors, equations (8) and (9), the following block diagram is obtained, see figure 2. A feedback connection is introduced through (6).

3. EXPERIMENTAL DATA

Data from two experiments using the probing feeding strategy described in (Åkesson *et al.*, 2001a) are used in the parameter estimation. For medium composition and equipment used, see (de Maré *et al.*, 2005). One of the experiments is shown in figure 3. As seen in the figure, linearisation around a stationary trajectory is necessary.

3.1 *Trajectories*

First the trajectories of the inputs are determined. Stationarity is assumed in the beginning of each pulse and F^o and N^o are adapted in a least-squares sense to these points. As the output DO_p is controlled to 30 % between the superimposed feed-pulses, $DO^o = 30$ %. To be able to calculate the trajectory of the output O_2^{an}, V^o,

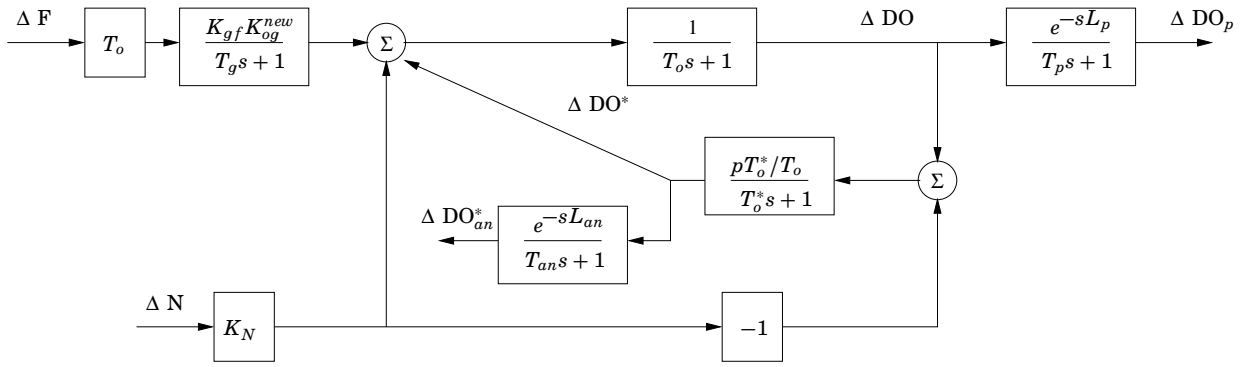

Fig. 2 Block diagram of the linearised process. The parameters are given in the appendix.

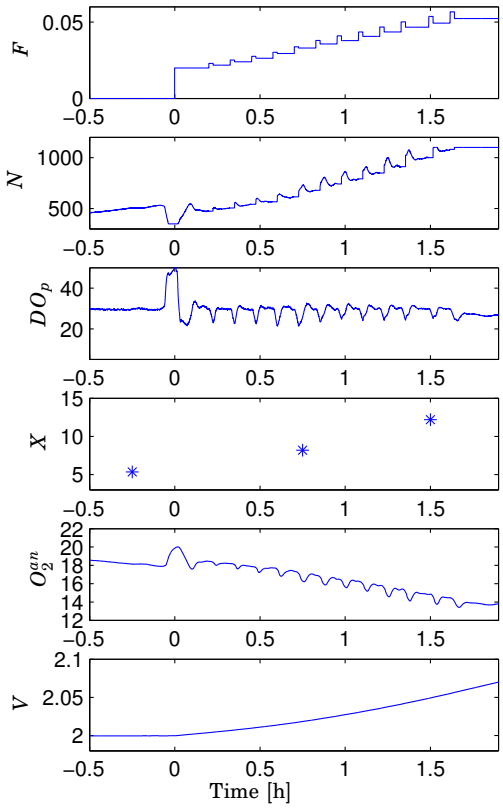

Fig. 3 Experimental data from cultivation 1. The region used for identification is from 0.2 h to 1.6 h where $q_o < q_o^{max}$ and no acetic acid is present. From the top: feed rate F [l/h], stirrer speed N [rpm], dissolved oxygen DO_p [%], cell mass concentration X [g/l], oxygen concentration in the outlet air O_2^{an} [%], reactor medium volum V [g/l]. Time after feed-start.

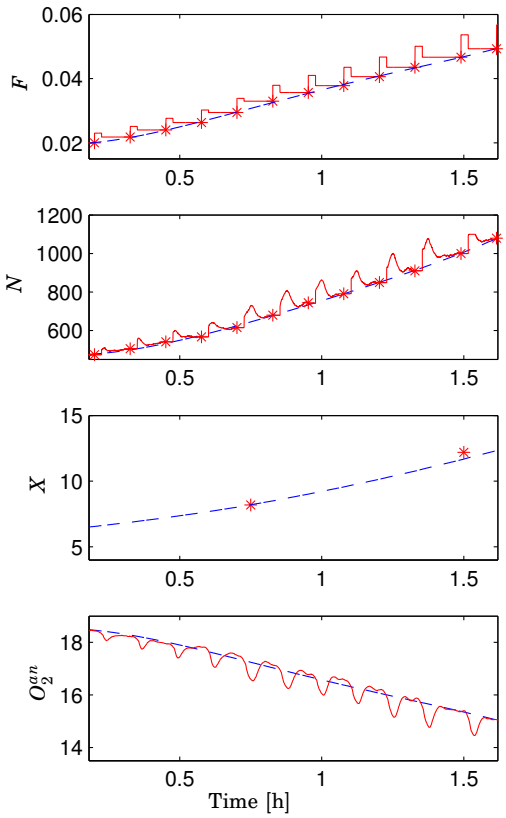

Fig. 4 Cultivation 1. The trajectories: feed rate F^o [l/h], stirrer speed N^o [rpm], cell mass concentration X^o [g/l] and oxygen concentration O_2^o [%] (dashed) together with experimental data (solid) are shown.

X^o and q_g^o are needed. The change in volume is small as seen in figure 3 and $V^o = V$. X^o is calculated from (4) where Y_{xg}^{ox}, Y_{og}^{ox}, q_m, Y^{om} are taken from (Xu *et al.*, 1999), see table 3. q_g^o is calculated from (2). The trajectory O_2^o is then determined using (5) and (6):

$$O_2^o = O_2^{in} - \frac{X^o RTV^o}{PQM}((q_g^o - q_m)Y_{og}^{ox} + q_m Y^{om})$$

Recalibration of the gas analyser is necessary in order to correlate O_2^{an} to O_2^o. In figure 4 and figure 5 F, N, X and O_2^{an} together with their trajectories are shown for cultivation 1 and cultivation 2, respectively.

4. PARAMETER ESTIMATION

As is seen in figure 2, there are 6 parameters to estimate, $K_{gf}K_{og}^{new}$, T_g, T_o, K_N, p, T_o^* from the bio-reactor and 4 parameters from the measurement equipment T_p, L_p, T_{an}, L_{an}. As this is not possible using the two sets of data available, we have to make some assumptions. Here we assume that the oxygen probe dynamics and the gas analyser dynamics are known. When examining the 6 parameters left we suspect T_o and

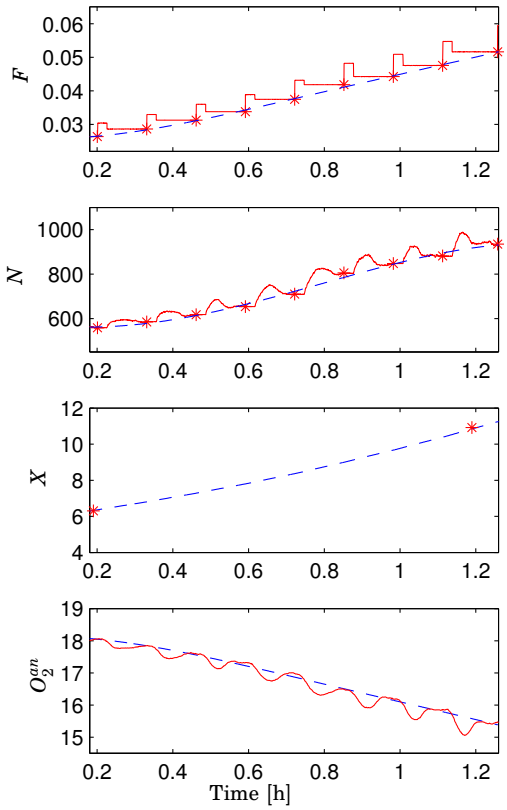

Fig. 5 Cultivation 2. The trajectories: feed rate F^o [l/h], stirrer speed N^o [rpm], cell mass concentration X^o [g/l] and oxygen concentration O_2^o [%] (dashed) together with experimental data (solid) are shown.

K_N to vary a lot. They depend on K_La^o and $\frac{\partial K_La}{\partial N}$, respectively, which change much during a cultivation. Therefore we calculate K_La^o using (6)

$$K_La^o = \frac{QPMH}{V^oRT}\frac{O_2^{in} - O_2^o}{DO^{*o} - DO^o}$$

and then $K_La(N)$ is calculated using K_La^o and N_o, see below. Thus we can determine $T_o = \frac{1}{K_La^o}$ and K_N. There are now four parameters left to identify which seems possible with the data available.

4.1 *Determination of K_N using $K_La(N)$*

To determine $K_La(N)$, a third order polynomial is chosen and its coefficients are adapted in a least-squares sense using N^o and K_La^o. The relation $K_La(N)$ is given by:

$$K_La(N) = \alpha_1 N^3 + \alpha_2 N^2 + \alpha_3 N + \alpha_4$$

The values of α are given in table 3 and their values differ for cultivation 1 and cultivation 2. In order to evaluate the expressions for $K_La(N)$, simulations with the non-linear model are shown in figure 6 and figure 7. The parameter values used are given in table 3.

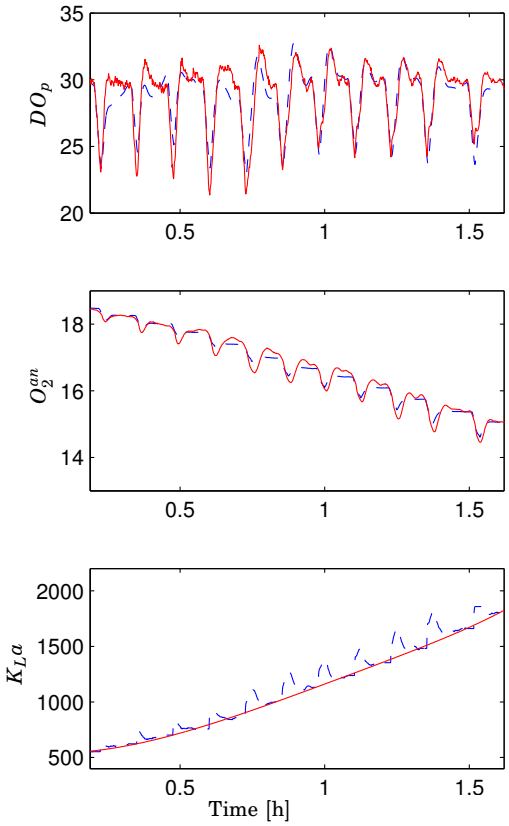

Fig. 6 Cultivation 1, simulation of the full model. From the top: dissolved oxygen DO_p^{sim} [%], oxygen concentration $O_{2,an}^{sim}$ [%], oxygen transfer $K_La(N)^{sim}$ [h^{-1}] (dashed) together with experimental data (solid) and K_La^o (solid).

4.2 *Adaptation*

The parameters left for estimation are: $K_{gf}K_{og}^{new}$, T_g, T_o^* and p. The minimisation criterion chosen is the cost-function V_{min}

$$\begin{aligned} V_{min} &= V_1 + V_2 \\ &= \Sigma(DO_p^{sim} - DO_p^{exp})^2 \\ &\quad + \Sigma(DO_{an}^{*,sim} - DO_{an}^{*,exp})^2 \end{aligned} \quad (10)$$

and the algorithm used is the Nelder-Mead simplex method. For adaptation, data from cultivation 1 are used. The starting values of the parameters are calculated from table 3 and are given in table 2 together with the obtained result from the minimization. For comparison purposes also the cost function for the non-linear simulation in figure 6 is presented. DO_p^{sim} and $DO_{sim}^{*,an}$ are shown together with the experimental data in figure 8.

4.3 *Validation*

For validation, data from cultivation 2 are used and the resulting DO_p^{sim} and $DO_{sim}^{*,an}$ obtained are shown in figure 9. The cost function for the validation and for the non-linear simulation in figure 7 is presented in table 2. To investigate

Table 2 Result of the parameter estimation. The data are sampled every 5 s. 1026 data points of cultivation 1 are used for adaptation and 907 data points of cultivation 2 are used for validation.

Data	Model	purpose	$K_{gf}K_{og}^{new}$	T_g [s]	T_o^* [s]	p	V_1	V_2	V_{min}
cult. 1	full model	-	-	-	-	-	1050	450	1500
cult. 1	linear mod.	start. values	$-1.45\cdot10^6$	12.6	20.4	0.0524	1505	585	2090
cult. 1	linear mod.	adaptation	$-1.43\cdot10^6$	13.4	17.5	0.0516	1290	490	1780
cult. 2	full model	-	-	-	-	-	865	55	920
cult. 2	linear mod.	validation	$-1.43\cdot10^6$	13.4	17.5	0.0516	840	120	960

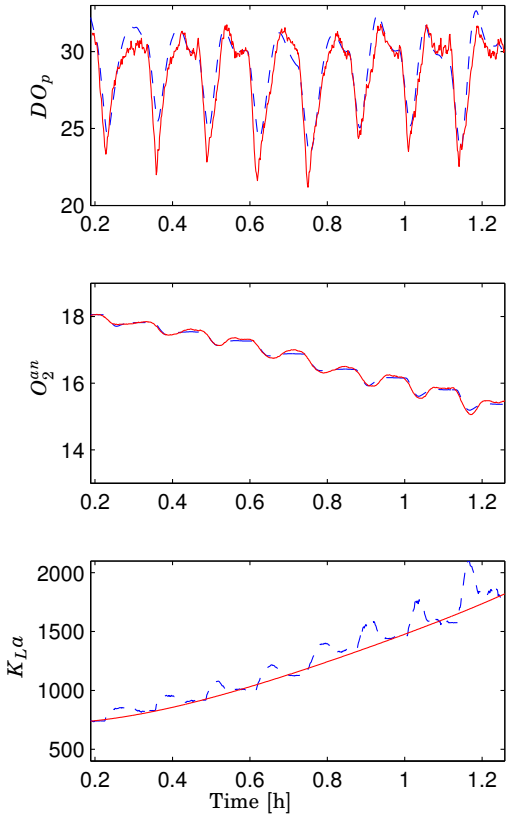

Fig. 7 Cultivation 2, simulation of the full model. From the top: dissolved oxygen DO_p^{sim} [%], oxygen concentration $O_{2,an}^{sim}$ [%], oxygen transfer $K_La(N)^{sim}$ [h^{-1}] (dashed) are shown together with experimental data (solid) and K_La^o (solid)

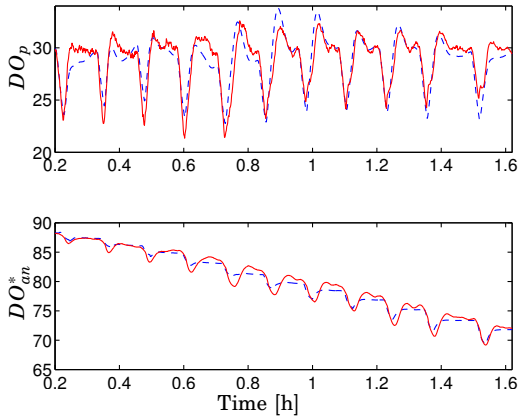

Fig. 8 Cultivation 1, adaptation of the linearised model. From the top: dissolved oxygen DO_p^{sim} [%], dissolved oxygen concentration in equilibrium with the outlet air $DO_{an}^{*,sim}$ [%] are shown (dashed) together with experimental data (solid).

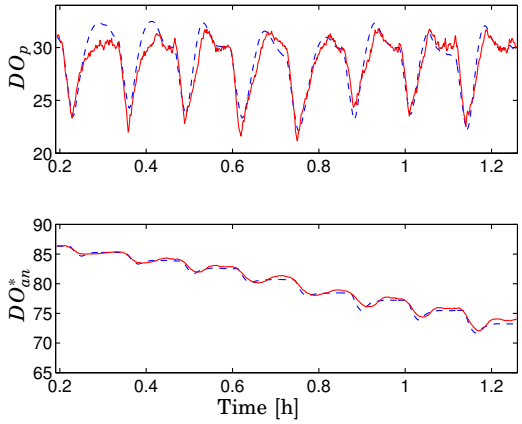

Fig. 9 Cultivation 2, validation of the linearised model. From the top: dissolved oxygen DO_p^{sim} [%], dissolved oxygen concentration in equilibrium with the outlet air $DO_{an}^{*,sim}$ [%] are shown (dashed) together with experimental data (solid).

the robustness of the obtained result more simulations are done with different parameter values. In these studies a strong correlation between p and T_o^* is noticed. As the value of p should not deviate much from the starting value as it contains well known physical parameters, we believe that the right minimum is found.

5. REVISED TUNING OF THE PROBING FEED CONTROLLER

When using a proportional probing feed controller the increase in the feed F is decided by

$$\Delta F(k) = \kappa \frac{DO_{pulse}(k) - y_r}{DO^* - DO^o} F \qquad (11)$$

where DO_{pulse} is the pulse response, y_r is the desired pulse response and κ the controller gain. There are several more parameters that need to be chosen such as the pulse duration T_{pulse}, the length between the pulses $T_{control}$ and the pulse height γ_p. In (Åkesson *et al.*, 2001b) some tuning rules are derived which we will examine here and modify if necessary. In our model the changing oxygen concentration in the outlet air is included which leads to additional dynamics

and a changed process gain.

The choice of T_{pulse} and $T_{control}$ depends on the process dynamics. In (Åkesson *et al.*, 2001b) T_{pulse} is chosen as a lumped time constant $T_{max} = T_p + T_o^{max} + T_g^{max}$ and $T_{control}$ is chosen to $4T_{pulse}$. Here a pulse response DO_{pulse} to a feed pulse F_p is given by

$$DO_{pulse} = \frac{K_{gf}|K_{og}|(T_o^* s + 1)e^{-sL_p}}{((T_o^* s + 1)(T_o s + 1) - p\frac{T_o^*}{T_o})(T_g s + 1)(T_p s + 1)} F_p$$

Thus as long as $p\frac{T_o^*}{T_o} \ll 1$ the guideline above still applies. Here $p\frac{T_o^*}{T_o}$ varies between 0.1-0.5, see table 2. Also, $T_o = \frac{1}{K_L a^o}$ and $K_L a^o$ is presented in figure 6 and figure 7. Considering the variation in $p\frac{T_o^*}{T_o}$ we suggest the use of $2T_o^*$ as the maximal lumped time constant for

$$\frac{T_o^* + 1}{(T_o^* s + 1)(T_o s + 1) - p\frac{T_o^*}{T_o}}$$

This gives a $T_{max} = T_p + T_g^{max} + 2T_o^* + L_p$ of approximately 110 s.

The choice of γ_p and κ depends on the process gain. When choosing γ_p it must be assured that the oxygen level does not become too low during a pulse which gives the upper limit. In steady state the amplitude of the oxygen response away from DO^o is given by:

$$DO_{pulse} = \frac{K_{gf}|K_{og}|}{1 - p\frac{T_o^*}{T_o}} F_p \leq \frac{DO^* - DO^o}{(1 - p\frac{T_o^*}{T_o})} \gamma_p$$

where $F_p = \gamma_p F$. Thus the upper limit for the value of γ_p is lower than in (Åkesson *et al.*, 2001b) where

$$DO_{pulse} \leq (DO^* - DO^o)\gamma_p$$

In (Åkesson *et al.*, 2001b) the controller gain $\kappa < 1$ ensures stability but in our case the corresponding requirement on κ is: $\kappa < 1 - p\frac{T_o^*}{T_o}$ which leads to a lower value of κ.

6. DISCUSSION

As is seen in figure 8 and figure 9, the linearised model seems to capture the behaviour well. Deviations are seen around 1 hour for cultivation 1 and in the beginning of cultivation 2. DO_{an}^* seems more difficult to adapt in cultivation 1. Also, note that different time constants T_{an} are used for the two cultivations, which can be explained by the fact that the behaviour of the gas analyser changes over time.

The time-variation in all the parameters T_g, T_o^*, $K_{gf}K_{og}^{new}$ and p is neglected, but even so there are not big differences in the results obtained when using the full model, see table 2. One explanation is that the time-variation in $K_{gf}K_{og}^{new}$ and p depends on the variation in V^o and V_g which is small, see figure 3. An investigation where the variations in T_g, depending on X and q_g^o, and in T_o^*, depending on T_o, are taken into account has been made, but the results are similar. Therefore the model with constant parameters seems suitable to use in the investigation of the feed controller tuning.

When it comes to the controller tuning, the equation describing the changing oxygen concentration in the outlet air makes a difference. A tighter upper bound on controller gain κ has to be satisfied to ensure stability. Also, when examining the feed controller described in equation (10) DO^* is included. In Åkesson's work DO^* is assumed to be constant. It will lead to a larger stationary error in the pulse responses than expected. To prevent this one can add the integral part to the feed controller, as is described in (Åkesson *et al.*, 2001b). An alternative is to make use of the measurements of the gas analyser, which are proportional to DO^*, as a gain scheduling variable.

In summary, a model describing the oxygen dynamics in a *E. coli* fed-batch cultivation is presented. In a linearised version the parameters are estimated and validated with good results. The model is used to discuss the guidelines on the feed controller tuning, derived in (Åkesson *et al.*, 2001b).

7. ACKNOWLEDGEMENT

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A. APPENDIX

A.1 *Uptake rates*

The glucose uptake rate q_g is given by:

$$q_g = \frac{q_g^{max} G}{k_s + G} \tag{12}$$

The glucose used for maintenance purposes is given by:

$$q_m = \min(q_g, q_{mc})$$

The glucose used for growth uptake is thus:

$$q_{gg} = q_g - q_m$$

Splitting into an oxidative flow and a fermentative flow gives:

$$q_{gg}^{ox} = \min((q_o^{max} - q_m Y^{om})/Y_{og}^{ox}, q_{gg})$$

$$q_{gg}^{fe} = q_{gg} - q_{gg}^{ox}$$

Specific acetate production q_a^p is given by:

$$q_{ap} = q_{gg}^{fe} Y_{ag}$$

Specific acetate consumption q_a^c :

$$q_{ac}^{pot} = q_{ac}^{max} A/(k_a + A)$$

$$q_{ac} = \min(q_{ac}^{pot}, (q_o^{max} - q_{gg}^{ox} Y_{og}^{ox} - q_m Y^{om})/Y_{oa})$$

The resulting acetate formation rate q_a:

$$q_a = q_{ap} - q_{ac}$$

Specific growth rate μ:

$$\mu = q_{gg}^{ox} Y_{xg}^{ox} + q_{gg}^{fe} Y_{xg}^{fe} + q_{ac} Y_{xa}$$

Specific oxygen uptake rate q_o is given by:

$$q_o = q_{gg}^{ox} Y_{og}^{ox} + q_m Y^{om} + q_{ac} Y_{oa}$$

A.2 *Linearised model*

The linearised parameters:

$$K_{og} = -\frac{HX^o}{K_L a^o} Y_{og}^{ox} \qquad T_g = (\frac{\partial q_g^o}{\partial G} X^o)^{-1}$$

$$K_{gf} = \frac{G_{in}}{V^o X^o} \qquad T_o = (K_L a^o)^{-1}$$

$$K_{gf} K_{og}^{new} = \frac{K_{gf} K_{og}}{T_o} = \frac{H Y_{og}^{ox} G_{in}}{V^o}$$

$$T_o^* = \frac{V_g^o}{V^o RT 100 K_L a^o + QMHPO_2^{in}}$$

$$K_N = \frac{DO^{*o} - DO^o}{K_L a^o} \frac{\partial K_L a}{\partial N}$$

$$K_{o^*o} = \frac{100 V^o RT K_L a^o}{V^o RT 100 K_L a^o + QMHPO_2^{in}} = \frac{pT_o^*}{T_o}$$

$$K_{o^*N} = -\frac{(DO^{*o} - DO^o) 100 V^o RT}{V^o RT 100 K_L a + QMHPO_2^{in}} \frac{\partial K_L a}{\partial N}$$

$$= -\frac{K_N p T_o^*}{T_o}$$

where $p = \frac{V^o RT 100}{PMHO_2^{in} V_g^o}$.

Table 3 Parameters in the model.

Symbol	Value	Unit	Description
$Vtot$	3	l	total reactor volume
R	8.314	J/(mol K)	ideal gas constant
T	22	^{o}C	air flow temperature
P	101.3	kPa	pressure
M	32	g/mol	oxygen molar mass
O_2^{in}	20.9	%	oxyg. conc. in the inlet air
G_{in}	500	g/l	glucose conc. in feed
H	14000	%l/g	Henrys const.
k_{sg}	0.01	g/l	saturation const. for glucose uptake
q_g^{max}	1.6	g/gh	max. spec. glucose uptake
q_o^{max}	0.6	g/gh	max. spec. oxygen uptake
q_{mc}	0.06	g/gh	maintenance coefficient
Y_{oa}	0.55	g/g	oxygen/acetate yield
Y_{og}^{ox}	0.414	g/g	oxygen/glucose yield for growth
Y^{om}	1.07	g/g	oxygen/glucose yield for maintenance
Y_{xa}	0.4	g/g	biomass/acetate yield
Y_{xg}^{ox}	0.51	g/g	oxidative biomass/glucose
Y_{xg}^{fe}	0.15	g/g	fermentative biomass/glucose
L_p	5	s	time delay dissolved oxygen sensor
T_p	60	s	time const. dissolved oxygen sensor
L_{an}	65	s	time delay gas analyser
T_{an}	0, 15	s	time const. gas analyser (cult. 1, cult 2)
Q	147, 161	l/h	air flow (cult. 1, cult. 2)
α_1	$5.9\cdot 10^{-8}$		oxygen transfer parameter (cult. 1)
α_2	$-3.7\cdot 10^{-4}$		oxygen transfer parameter (cult. 1)
α_3	2.6		oxygen transfer parameter (cult. 1)
α_4	-581		oxygen transfer parameter (cult. 1)
α_1	$1.9\cdot 10^{-5}$		oxygen transfer parameter (cult. 2)
α_2	-0.041		oxygen transfer parameter (cult. 2)
α_3	31		oxygen transfer parameter (cult. 2)
α_4	-7160		oxygen transfer parameter (cult. 2)