# 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

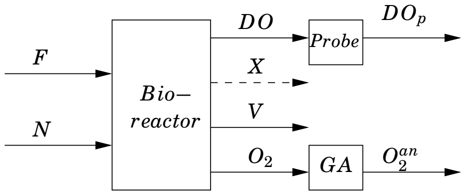


**Fig. 1** Block diagram of the process. $DO$ dissolved oxygen concentration, $F$ feed rate, $N$ stirrer speed, $V$ the reactor medium volume, $X$ cell mass concentration, GA = gas analyser, probe = dissolved oxygen probe.

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 $\mu$, 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 $O_2^{an}$, $V^o$,

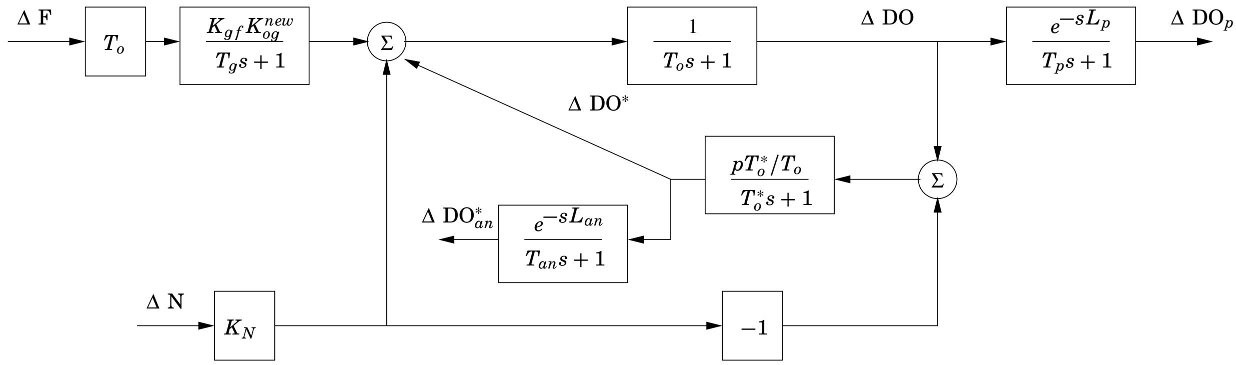


**Fig. 2** Block diagram of the linearised process. The parameters are given in the appendix.

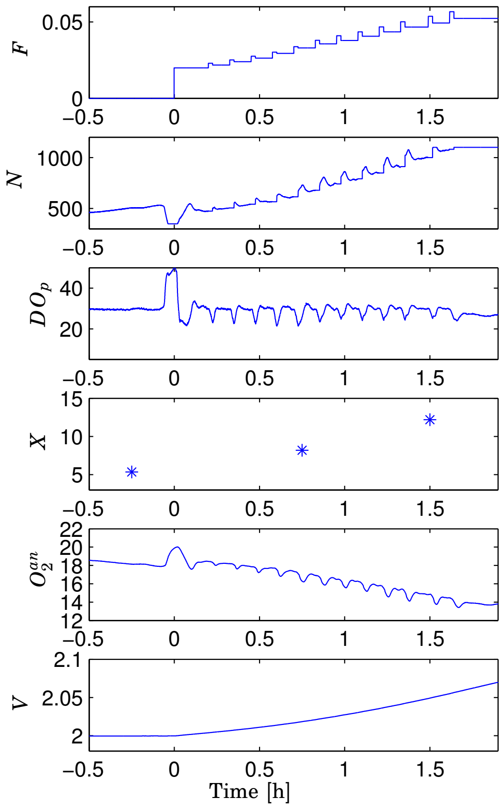


**Fig. 3** Experimental data from cultivation 1. The region used for identification is from 0.2 h to 1.6 h where $q_o < q_o^{max}$ and no acetic acid is present. From the top: feed rate $F$ [l/h], stirrer speed $N$ [rpm], dissolved oxygen $DO_p$ [%], cell mass concentration $X$ [g/l], oxygen concentration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

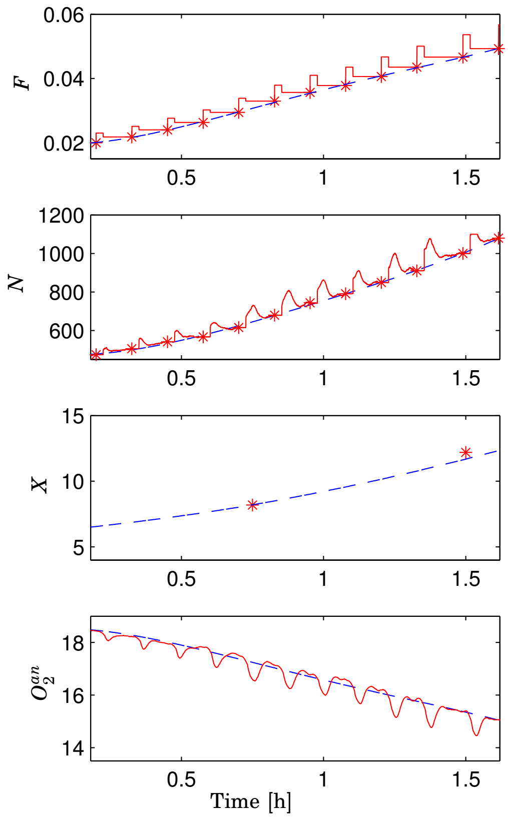


**Fig. 4** Cultivation 1. The trajectories: feed rate $F^o$ [l/h], stirrer speed $N^o$ [rpm], cell mass concentration $X^o$ [g/l] and oxygen concentration $O_2^o$ [%] (dashed) together with experimental data (solid) are shown.

$X^o$ 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

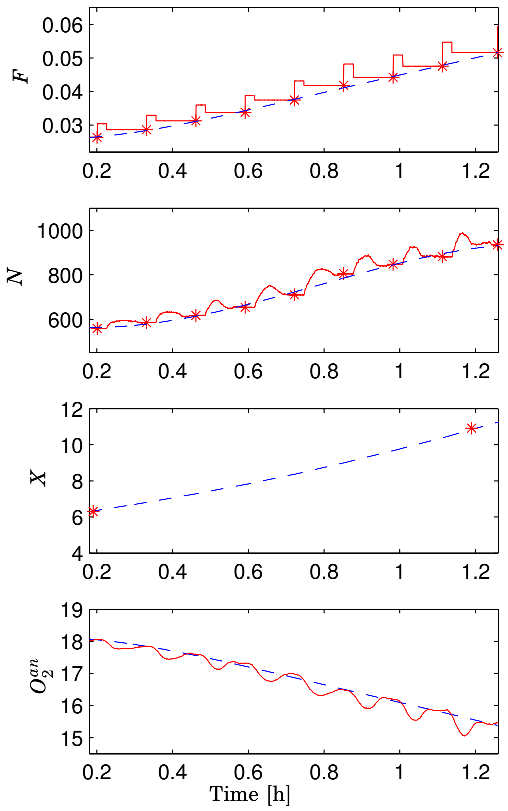


**Fig. 5** Cultivation 2. The trajectories: feed rate $F^o$ [l/h], stirrer speed $N^o$ [rpm], cell mass concentration $X^o$ [g/l] and oxygen concentration $O_2^o$ [%] (dashed) together with experimental data (solid) are shown.

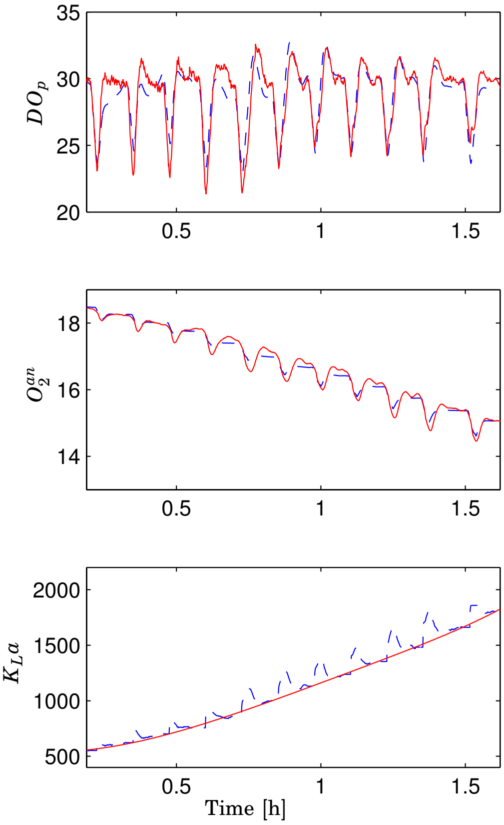


**Fig. 6** Cultivation 1, simulation of the full model. From the top: dissolved oxygen $DO_p^{sim}$ [%], oxygen concentration $O_{2,an}^{sim}$ [%], oxygen transfer $K_La(N)^{sim}$ [h$^{-1}$] (dashed) together with experimental data (solid) and $K_La^o$ (solid).

$K_N$ 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 $\alpha$ 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.

### 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 $V_{min}$

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

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 | -1.45·10^6 | 12.6 | 20.4 | 0.0524 | 1505 | 585 | 2090 |
| cult. 1 | linear mod. | adaptation | -1.43·10^6 | 13.4 | 17.5 | 0.0516 | 1290 | 490 | 1780 |
| cult. 2 | full model | - | - | - | - | - | 865 | 55 | 920 |
| cult. 2 | linear mod. | validation | -1.43·10^6 | 13.4 | 17.5 | 0.0516 | 840 | 120 | 960 |

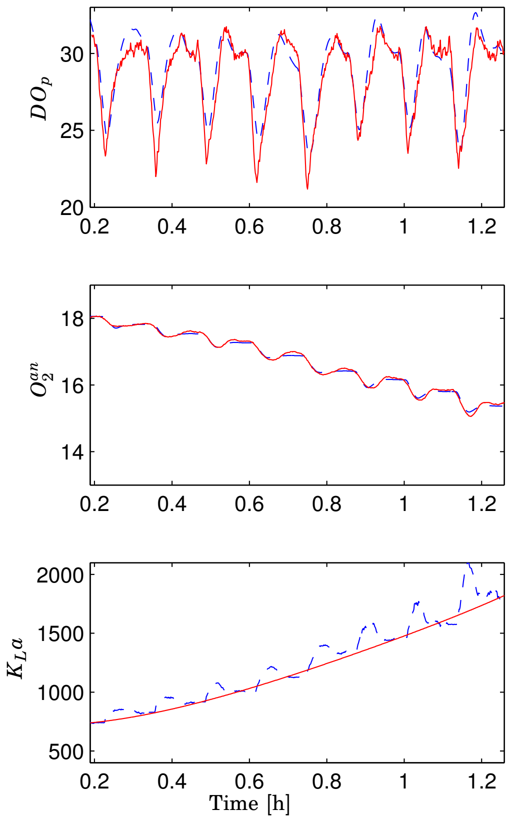

**Fig. 7** Cultivation 2, simulation of the full model. From the top: dissolved oxygen $DO_p^{sim}$ [%], oxygen concentration $O_{2,an}^{sim}$ [%], oxygen transfer $K_La(N)^{sim}$ [h$^{-1}$] (dashed) are shown together with experimental data (solid) and $K_La^o$ (solid)

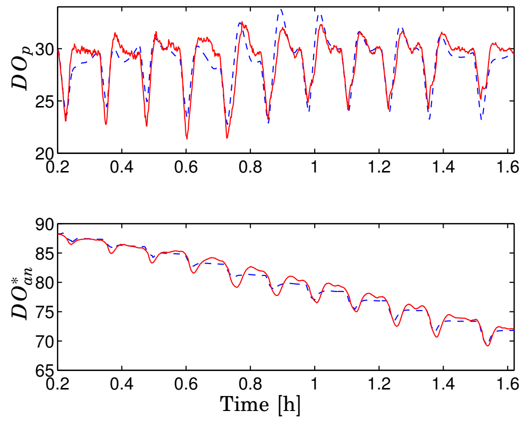

**Fig. 8** Cultivation 1, adaptation of the linearised model. From the top: dissolved oxygen $DO_p^{sim}$ [%], dissolved oxygen concentration in equilibrium with the outlet air $DO_{an}^{*,sim}$ [%] are shown (dashed) together with experimental data (solid).

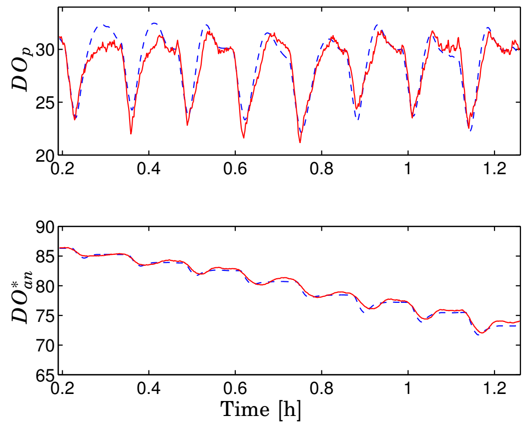

**Fig. 9** Cultivation 2, validation of the linearised model. From the top: dissolved oxygen $DO_p^{sim}$ [%], dissolved oxygen concentration in equilibrium with the outlet air $DO_{an}^{*,sim}$ [%] are shown (dashed) together with experimental data (solid).

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 $\kappa$ 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 $\gamma_p$. 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 $\gamma_p$ and $\kappa$ depends on the process gain. When choosing $\gamma_p$ 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:

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

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

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

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

## 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 $\kappa$ 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 $\mu$:

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

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:

$$K_{og} = -\frac{HX^o}{K_L a^o} Y_{og}^{ox} \qquad T_g = \left(\frac{\partial q_g^o}{\partial G} X^o\right)^{-1}$$
$$K_{gf} = \frac{G_{in}}{V^o X^o} \qquad T_o = (K_L a^o)^{-1}$$

$$\begin{aligned}
K_{gf} K_{og}^{new} &= \frac{K_{gf} K_{og}}{T_o} = \frac{H Y_{og}^{ox} G_{in}}{V^o} \\
T_o^* &= \frac{V_g^o}{V^o RT 100 K_L a^o + QMHPO_2^{in}} \\
K_N &= \frac{DO^{*o} - DO^o}{K_L a^o} \frac{\partial K_L a}{\partial N} \\
K_{o^*o} &= \frac{100 V^o RT K_L a^o}{V^o RT 100 K_L a^o + QMHPO_2^{in}} = \frac{pT_o^*}{T_o} \\
K_{o^*N} &= -\frac{(DO^{*o} - DO^o) 100 V^o RT}{V^o RT 100 K_L a + QMHPO_2^{in}} \frac{\partial K_L a}{\partial N} \\
&= -\frac{K_N p T_o^*}{T_o}
\end{aligned}$$

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) |
| $\alpha_1$ | $5.9\cdot 10^{-8}$ | | oxygen transfer parameter (cult. 1) |
| $\alpha_2$ | $-3.7\cdot 10^{-4}$ | | oxygen transfer parameter (cult. 1) |
| $\alpha_3$ | 2.6 | | oxygen transfer parameter (cult. 1) |
| $\alpha_4$ | -581 | | oxygen transfer parameter (cult. 1) |
| $\alpha_1$ | $1.9\cdot 10^{-5}$ | | oxygen transfer parameter (cult. 2) |
| $\alpha_2$ | -0.041 | | oxygen transfer parameter (cult. 2) |
| $\alpha_3$ | 31 | | oxygen transfer parameter (cult. 2) |
| $\alpha_4$ | -7160 | | oxygen transfer parameter (cult. 2) |