Control of Dissolved Oxygen in Stirred Bioreactors

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1998

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Abstract This report discusses control of dissolved oxygen in a bioreactor where the oxygen supply is manipulated using the stirrer speed. In batch and fed-batch cultivations the operating conditions change significantly which may cause tuning problems. Analysis using a linearized process model shows that the process dynamics is mainly affected by changes in the volumetric oxygen transfer coefficient $K_L a$. To account for the process variations, a control strategy based on PID control and gain scheduling from the stirrer speed is suggested. This approach is straightforward to implement in an industrial control system. Experimental results from a laboratory reactor are presented.

Keywords Feedback control, dissolved oxygen, stirrer speed, bioreactor, gain scheduling.

1. Introduction

Today, many commercial products are produced using microorganisms. Living cells are grown to large numbers and made to produce a desired substance, often a protein. The cells are kept in a bioreactor where several control loops ensure that important process parameters, such as pH and temperature, stay within specified operating conditions. In aerobic processes, it is also important to provide the culture with oxygen. This is often solved by keeping a constant dissolved oxygen concentration.

Figure 1 shows a drawing of a stirred bioreactor. In the reactor there is a liquid medium that contains cells and substrates. Air is sparged into the liquid and well-mixed conditions are obtained through agitation with a mechanical stirrer. The oxygen supply can be varied by manipulating air flow rate, oxygen content in the incoming air, reactor pressure, and stirrer speed. In this report, we will discuss how to control dissolved oxygen using the stirrer speed as control signal.

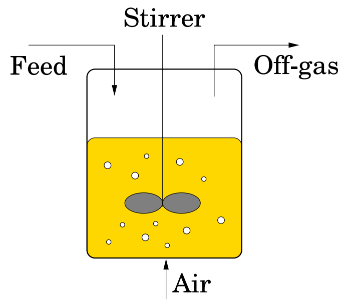

Figure 1 Stirred bioreactor.

1.1 Control Problem

When controlling dissolved oxygen in a bioreactor, it is often sufficient to keep the dissolved oxygen above a certain level. In some applications the performance requirements are higher, for instance when elevated oxygen levels are toxic for the microorganisms. Tight control is a key element in the substrate-dosage control scheme presented in [Åkesson *et al.*, 1997] and it may also be beneficial for process supervision. If the dissolved oxygen level is kept constant, the control input can be used as an indicator of the biological activity, see for instance [Lee *et al.*, 1996].

From a control point of view, dissolved oxygen control is a regulation problem where the main disturbance is the oxygen consumption due to cell metabolism. Other disturbances affecting the process include temperature changes, foaming, and addition of surface active components. At low turbulence levels, measurement errors may occur due to gas bubbles that adhere to the sensor surface, see [Heinzle *et al.*, 1986]. In [Yano *et al.*, 1981], a classification of these disturbances is used to derive a rule-based control strategy. Controllers of PI-, and PID-type, where the parameters are obtained from open loop experiments, have for instance been presented in [Court, 1988] and [Clark *et al.*, 1985].

In continuous cultivations, the process is operated at steady-state conditions and good performance can be expected using controllers with fixed parameters. However, when the reactor is run in batch or fed-batch mode, important process variables, e.g., cell mass, substrate concentration, oxygen uptake etc., are no longer constant and the process characteristics may vary significantly. Many authors have reported tuning difficulties when controllers with fixed parameters are used, see [Court, 1988], [Lee *et al.*, 1991] and [Cardello and San, 1988]. Typical observations are stability problems for low uptake rates and sluggish control at high uptake rates. Similar difficulties occur in dissolved control of activated sludge processes in wastewater treatment, where the air flow rate is used as control signal, see for instance [Lindberg, 1997] and [Holmberg *et al.*, 1989].

To overcome the problems with the varying process dynamics, various adaptive control schemes have been suggested. Approaches based on PID control together with gain scheduling from the oxygen uptake rate and PID control combined with feed-forward from the oxygen uptake rate were presented in [Cardello and San, 1988]. On-line tuning of PID controllers based on output variance [Lee *et al.*, 1992] and parameter estimation from off-gas analysis [Levisauskas, 1995] have also been suggested. Indirect adaptive control, that is, parameter estimation and subsequent controller design, have been tested in [Lee *et al.*, 1991] and [Hsiao *et al.*, 1992].

1.2 Outline

The outline of the report is as follows. In Section 2, a model of the oxygen dynamics is derived. Analysis of a linearized model shows that the oxygen dynamics depends critically on the volumetric oxygen transfer coefficient $K_L a$. The implications for control are discussed in Section 3. A control strategy that is straightforward to implement in an industrial process control system is suggested. Section 4 presents experimental results from dissolved oxygen control in a laboratory scale bioreactor during a cultivation of recombinant *Escherichia coli* bacteria. Finally, in Section 5 some conclusions are given.

2. Process Model

A model of the dissolved oxygen dynamics in a laboratory scale bioreactor will be derived. It is assumed that well-mixed conditions apply and that any mechanical or electrical dynamics from control signal to stirrer speed are negligible. The parameters in the model are adjusted to describe a 3 liter laboratory bioreactor according to experimental data. A linearized model is derived for analysis purposes.

2.1 Nonlinear Model

Oxygen balance Mass-balance for the dissolved oxygen in the reactor yields the following differential equation

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

where V, C_o, X, denote liquid volume, dissolved oxygen concentration, and cell concentration, respectively. The first term models the oxygen transfer from air bubbles to liquid. Here, K_La is the volumetric oxygen transfer coefficient which is dependent on the stirrer speed, N, and C_o^* denotes the oxygen concentration in the liquid layer around the air bubbles. The second term describes the oxygen consumption due to the biological activity. The specific oxygen consumption rate q_o is a function of substrate uptake rate and hence it will depend on the substrate feed rate.

Contributions from incoming and outgoing flows have been neglected because the oxygen solubility in water is very low. A similar argument can be used to neglect concentration due to dilution effects and the oxygen equation can be rewritten as

$$\frac{dC_o}{dt} = K_La(N) \cdot (C_o^* - C_o) - q_o \cdot X$$

In practice, most sensors do not measure the oxygen concentration but the dissolved oxygen tension, a quantity proportional to the oxygen partial pressure. A dissolved oxygen tension of 100 % corresponds to a solution where the oxygen partial pressure is in equilibrium with air, that is, an oxygen saturated solution. The dissolved oxygen tension O is related to the dissolved oxygen concentration through Henry's law

$$O = H \cdot C_o$$

where the constant H depends on the oxygen solubility, see [Popović *et al.*, 1979] and [Pirt, 1975]. In the sequel, the common literature value for water $H = 14000\,\%\,\mathrm{l\,g^{-1}}$ will be used. In a laboratory reactor it is also reasonable to assume that O^* is close to 100 %. The oxygen dynamics can now be described as

$$\frac{dO}{dt} = K_La(N) \cdot (O^* - O) - \underbrace{q_o \cdot HX}_{=d}$$

From now on, the oxygen consumption term will be considered as a load disturbance d.

Volumetric oxygen transfer coefficient For a fixed air flow rate, the volumetric oxygen transfer coefficient, K_La, can be modeled as a function of the stirrer speed, N. Commonly used expressions are of the form $K_La \sim N^\gamma$, for instance $K_La \sim N^3$ as suggested in [Pirt, 1975]. To obtain good mixing, the stirrer speed is in practice never below a minimum value. In the working range, it is then reasonable to approximate the stirrer dependence with a linear expression

$$K_La(N) = \alpha \cdot (N - N_0)$$

For the 3 l laboratory bioreactor, $\alpha = 0.92\ \mathrm{h}^{-1}\mathrm{rpm}^{-1}$ and $N_0 = 323$ rpm give a good approximation for stirrer speeds between 350 rpm to 1200 rpm. These values were obtained using the K_La-estimation technique suggested in [Van't Riet, 1979] for different stirrer speeds. Calculating the oxygen transfer rate, OTR, from off-gas analysis, K_La can be estimated as

$$K_La = \frac{H \cdot OTR}{O^* - O}$$

Except for air flow rate and stirrer speed, the oxygen transfer is also affected by viscosity, temperature, foaming etc., [Pirt, 1975]. Addition of antifoam chemicals also tend to give a temporary decrease in K_La.

Dissolved oxygen sensor Dissolved oxygen tension is measured with a probe in the reactor liquid. As will be seen, the probe contributes significantly to the oxygen dynamics. For large deviations, the probe tends to respond faster for up responses than for down responses, [Lee and Tsao, 1979]. The sensitivity of the probe does also increase with temperature. In large reactors, where mixing problems cannot be neglected, the probe placement may be important for the oxygen control performance [Belfares *et al.*, 1989].

For non-viscous and well-mixed systems the probe may be modeled as a linear first order system and possibly a time delay

$$T_p \frac{dO_p}{dt} + O_p(t) = O(t - \tau)$$

with O_p denoting the measured dissolved oxygen tension. However, for viscous systems and for low stirrer speeds, it may be necessary to add a second time constant, see [Dang *et al.*, 1977].

For the probe used in the laboratory reactor a time constant of $T_p \approx 20$ s was estimated. The time delay was less than the data logging period of 2 s. In the sequel $\tau = 2$ s will be used.

2.2 Linearized Model

Around a trajectory, small variations in the dissolved oxygen dynamics may be described by the linearized equation

$$T_o \frac{d\Delta O}{dt} + \Delta O = K_n \Delta N - K_d \Delta d$$

where the parameters are given by

$$\begin{aligned} T_o &= (K_La)^{-1} \\ K_n &= [O^* - O] \frac{\partial K_La}{\partial N} (K_La)^{-1} \\ K_d &= (K_La)^{-1} \end{aligned}$$

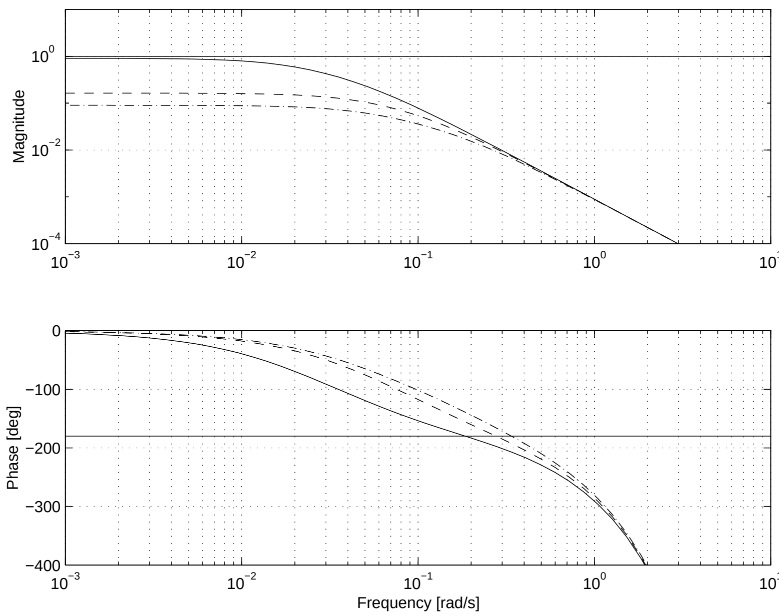

Figure 2 Bode plot of the transfer function from stirrer speed to measured dissolved oxygen tension at three different stirrer speeds; 400 rpm (solid), 750 rpm (dashed), 1100 rpm (dash-dotted).

The transfer function from the stirrer speed ΔN to the oxygen measurement ΔO_p becomes

$$G_{on}(s) = \frac{K_n e^{-s\tau}}{(1 + sT_o)(1 + sT_p)}$$

Here τ and T_p depend on the probe and are considered to be constant during a process run. On the other hand, K_n and T_o are both proportional to the inverse of $K_L a$ which may vary significantly. In Figure 2, a Bode plot of G_{on} at three different stirrer speeds illustrate the changing process dynamics. When $K_L a$ increases with the stirrer speed, K_n and T_o decrease which will decrease the low-frequency gain and increase the phase in the low- and mid-frequency region. The high-frequency region is unaffected.

In summary, the lower the $K_L a$, the higher is the process gain and the larger is the phase lag. Note also that the gain K_n depends on the linearization point in dissolved oxygen, i.e., the chosen set-point.

3. Control Design

The process model derived in the previous section will now be used to discuss the dissolved oxygen control problem. Design and analysis of controllers of PID-type are made based on the linearized model. The non-linear model is used for simulations of the closed-loop systems, see Figure 3. A control strategy based on gain scheduling from the stirrer speed is suggested.

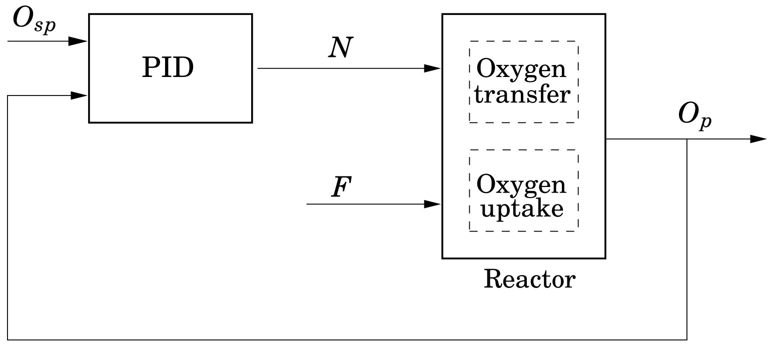

Figure 3 Dissolved oxygen control loop where the dissolved oxygen signal O_p should be kept at the set-point O_{sp}. By manipulating the stirrer speed N the oxygen transfer to the reactor can be varied. In fed-batch and continuous cultivations, the oxygen consumption depends on the substrate feed rate F.

3.1 Fixed-parameter Controller

We will now examine the behavior of controllers with fixed parameters when the process dynamics change. Using the Kappa-Tau tuning method, see [Åström and Hägglund, 1995], two PID controllers are designed to work well at 400 and 1100 rpm respectively. The resulting controller parameters can be found in Table 1. In both cases, low-pass filtering of the derivative term with a time constant $T_d/5$ is used.

Figure 4 shows the open-loop Bode diagram when the two controllers are operated around 400 rpm. The controller designed to work at 1100 rpm, which has higher gain and more integral action, seems to give a closed-loop system that is close to instability. This is confirmed when simulating the two systems, see Figure 5. The controller designed for 1100 rpm gives an oscillating system while the controller designed for 400 rpm gives well damped system that responds quickly to a step load change.

Around 1100 rpm, the process gain has decreased and both controllers give a well damped closed-loop system, see Figure 6. From the Bode diagram, it can also be seen that the controller designed for 1100 rpm gives

	K	T_i	T_d
400 rpm	12.9 rpm/%	24.6 s	5.7 s
1100 rpm	48.1 rpm/%	12.5 s	3.0 s

Table 1 PID parameters for the laboratory reactor using the Kappa-Tau tuning method with $M_s = 1.4$.

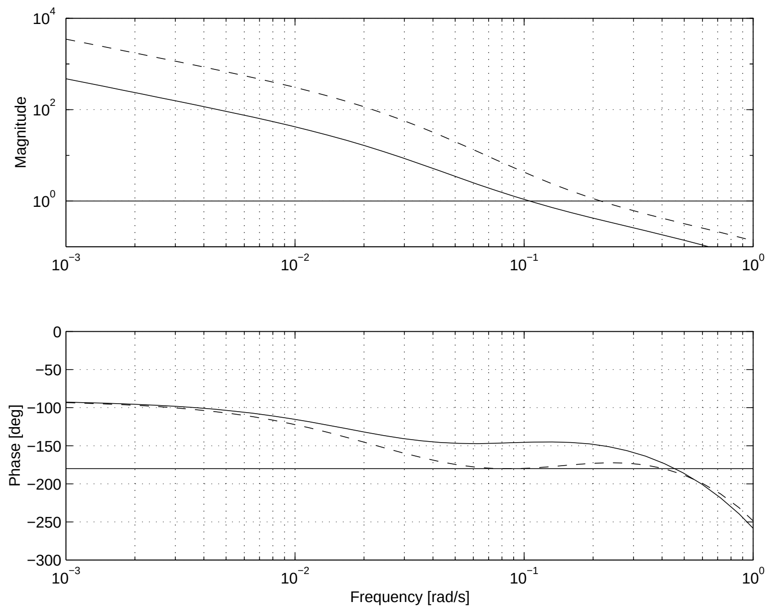

Figure 4 Bode plot of the open-loop transfer function at 400 rpm. PID controller tuned for 400 rpm (solid) and 1100 rpm (dashed).

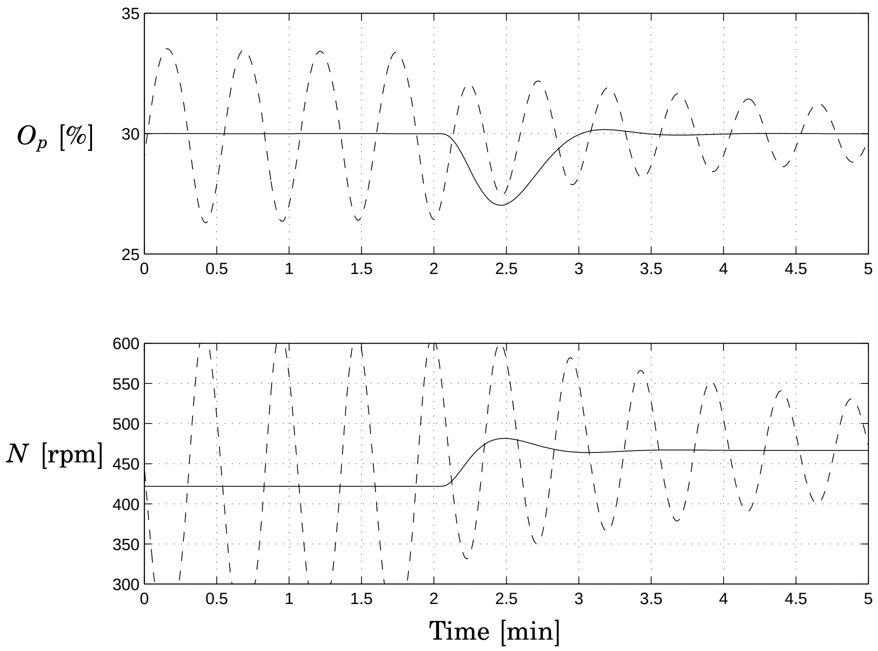

Figure 5 Closed-loop simulation around 400 rpm. After two minutes, a step change in the load is made. Results with PID controller tuned for 400 rpm (solid) and 1100 rpm (dashed).

a faster system with better load disturbance rejection. Again, this is confirmed when the closed-loop systems are simulated, see Figure 7. The controller designed for 1100 rpm is considerably faster without being oscillatory.

Thus, when a controller with fixed parameters is used, there is a trade-off between stability and performance. If stability in the presence of process

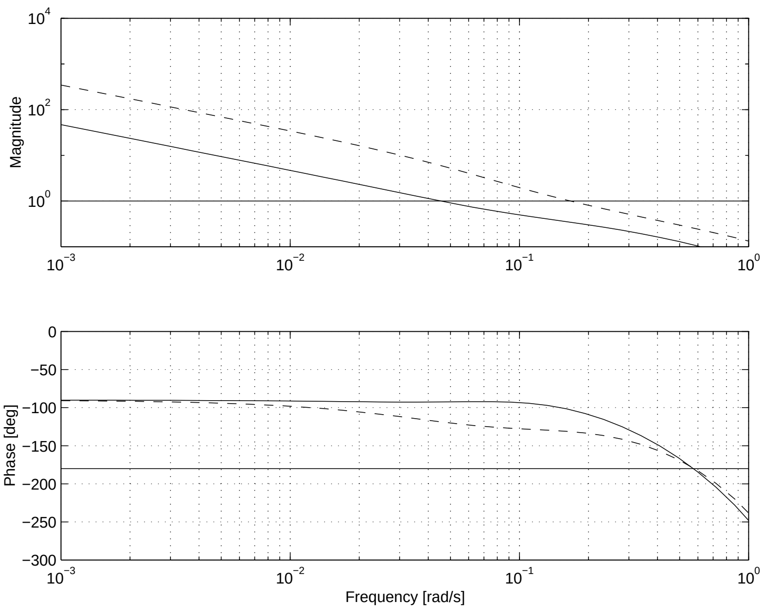

Figure 6 Bode plot of the open-loop transfer function at 1100 rpm. PID controller tuned for 400 rpm (solid) and 1100 rpm (dashed).

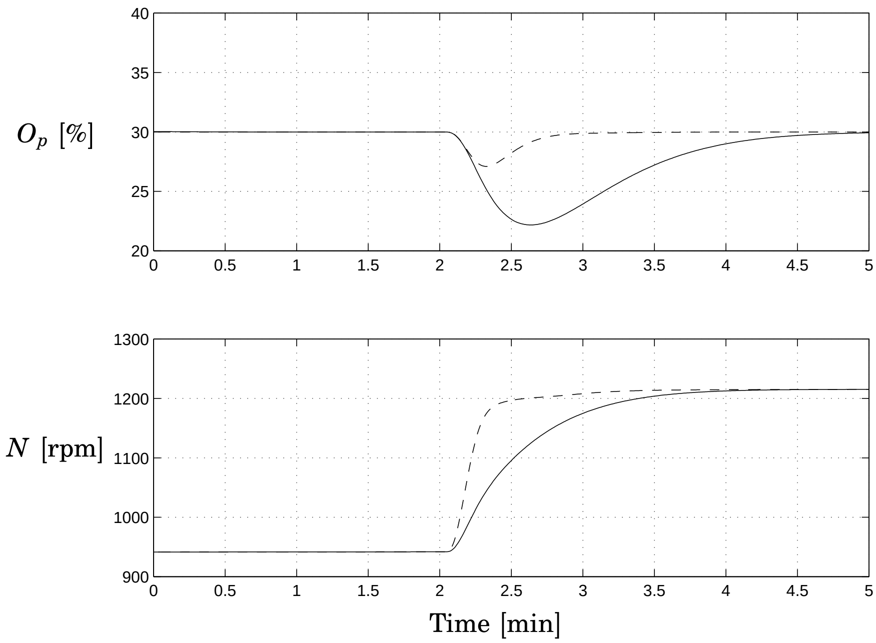

Figure 7 Closed-loop simulation around 1100 rpm. After two minutes, a step change in the load is made. Results with PID controller tuned for 400 rpm (solid) and 1100 rpm (dashed).

variations is the most important objective, the tuning should be made for the lowest K_La that is expected, i.e., at the lowest stirrer speeds. This gives a robustly stable closed-loop system at the expense of a sluggish response at higher K_La values.

It was pointed out that the K_La variations do not affect the high-frequency region. A high-gain feedback controller with fixed parameters could then, in principle, yield a closed-loop system insensitive to the variations. However, in practice this alternative is not feasible. For instance, the influence of measurement noise would be too large.

As pointed out in Section 2, the chosen set-point O_{sp} also affects the process behavior. If a large span of set-points are to be used this may have to be accounted for. Good robustness is obtained if the controller is tuned at the lowest set-point where the process gain is highest.

3.2 Gain Scheduling

To obtain the same performance at all operating points, without trading off robustness, the controller should depend on the operating conditions. One way to do this is to use gain scheduling [Åström and Hägglund, 1995]. As the process dynamics changes with K_La, this is a natural scheduling variable. An alternative and a slight variation of gain scheduling, is to use exact linearization, see [Lindberg, 1997]. Both methods require on-line estimation of K_La, which for instance can be done using off-gas measurements.

As K_La is dependent on the stirrer speed, a simpler approach is to use gain scheduling from the stirrer speed itself. This method is straightforward to implement in an industrial control system and will be used in the experiments in the next section. A drawback is that this technique does not capture K_La changes due to other sources than the stirrer speed, e.g., foaming, surface active compounds, viscosity changes etc. In processes where such effects are important, a method based on estimation of K_La is preferable.

3.3 Feed-forward

In fed-batch and continuous cultivations, the oxygen consumption is dependent on the substrate feed rate. Feed-forward action from the feed rate can then be used to improve the control performance. For instance, in fed-batch cultivations with exponential growth, the oxygen consumption increase exponentially. This kind of disturbance is hard to reject for an ordinary PID controller without feed-forward.

The dynamics from feed rate to oxygen consumption is often unknown but a static relation between feed rate and stirrer speed can be obtained from recorded process data.

4. Experiments

This section describes dissolved oxygen control in a 3 liter laboratory fermenter during fed-batch fermentations of recombinant *Escherichia coli*.

4.1 Control Strategy and Implementation

As was suggested in the previous section, a PID controller combined with gain scheduling from the stirrer speed was used. The working range for the stirrer was divided into three regions; 350–600 rpm, 600–900 rpm, and 900–1200 rpm. A hysteresis of ± 20 rpm was introduced to avoid oscillations around the transition regions when changing from one region to another. No feed-forward action was used during the experiments.

The controller was implemented in the SattLine system from Alfa Laval Automation AB, Malmö, Sweden. A standard module for PID control with facilities for gain scheduling and auto-tuning was used. Anti-reset windup and bumpless parameter changes are also included in the module. When derivative action was used, the derivative term was low pass filtered with a time constant $T_d/6$. A sampling time of 0.5 s was chosen according to the guidelines in [Åström and Wittenmark, 1997]. Limitations in the choice of sampling interval may impose restrictions on achievable performance, especially when derivative action is needed.

For each of the operating regions, the auto-tuner function in the controller was used to obtain controller parameters. The load was varied by changing the feed rate F. A constant feed rate gives an approximately constant oxygen consumption. Relay experiments made at the end of a cultivation are shown in Figure 8. The resulting controller parameters are given in Table 2.

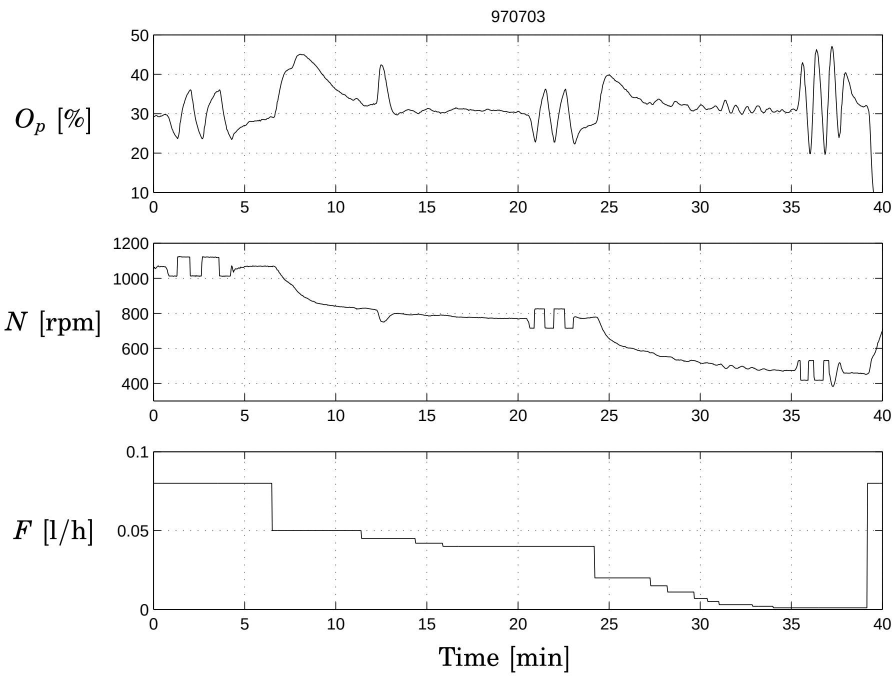

Figure 8 Relay experiments at three different operating points. The load has been varied by changing the feed rate F.

	K	T_i	T_d
450 rpm	3.2 rpm/%	29.4 s	4.7 s
750 rpm	6.3 rpm/%	40.8 s	0.0 s
1050 rpm	6.8 rpm/%	61.2 s	0.0 s

Table 2 PID parameters for the laboratory reactor obtained from relay auto-tuning experiments.

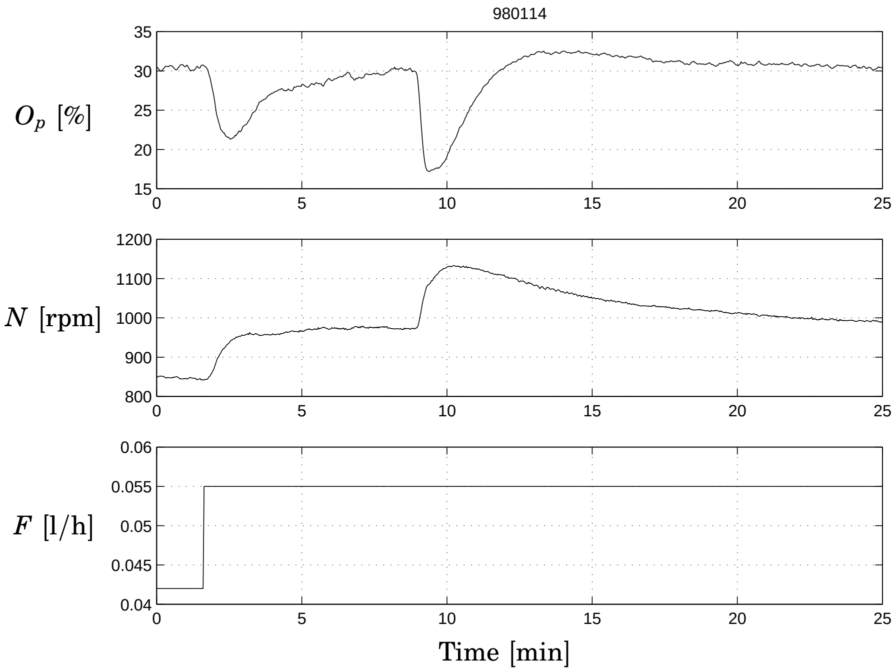

Figure 9 Step load change followed by anti-foam addition. At 920 rpm, the controller changed from the mid to the high operating region. The set-point O_{sp} was fixed at 30 %.

4.2 Results

The control algorithm was tested using the same reactor but with another strain of *E. coli*. In the mid operating region, the controller parameters obtained from the relay experiments gave a behavior that was somewhat oscillatory. The derivative time T_d was therefore increased from 0.0 s to 1.0 s. Load response experiments were made at the end of the cultivation. At this stage, there was substantial foaming in the reactor and anti-foam chemicals which affect the oxygen transfer had to be added several times.

In Figure 9, the behavior at higher stirrer speeds is shown. First, a step increase was made in the substrate feed rate F. This gives an approximate step change in the oxygen consumption. During this experiment, the controller changed from the mid to the high operating region. After about 9 minutes, an anti-foam addition was made which immediately reduced the oxygen transfer and the dissolved oxygen level O_p dropped. The controller compensated for this by increasing the stirrer speed N. The effect of the anti-foam on the oxygen transfer decayed slowly, which caused O_p to stay slightly above the set-point O_{sp} for a while. This can be thought of as the response to a ramp disturbance.

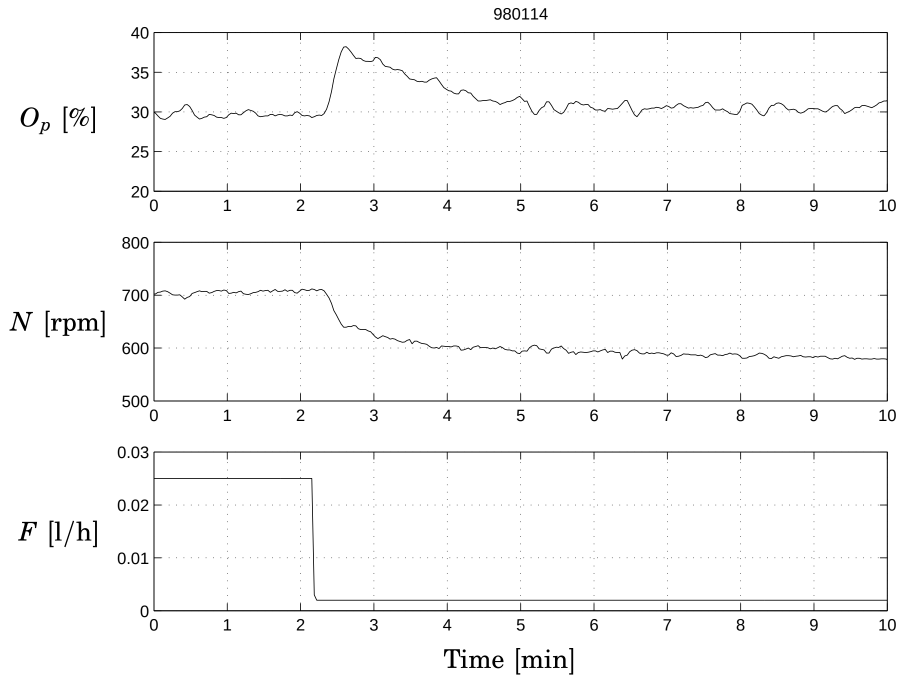

Figure 10 Step load change in the lower part of the mid operating region. The low region was never entered. The set-point O_{sp} was 30 %.

Figure 10 illustrates the response to a load change in the lower part of the mid operating region. The low region was never entered. The disturbance was eliminated faster than in the previous experiments but the variations in the output were substantially larger. Small damped oscillations can be hinted at. One explanation for this could be that the mid-controller was approaching the lower limit for its applicability. There also seemed to be more excitation from measurement "noise", possibly due to gas bubbles adhering to the sensor surface, see [Heinzle *et al.*, 1986].

The experiments indicate that the approach with gain scheduling from the stirrer speed works well. Good disturbance rejection was achieved throughout the operating range. However, some tuning problems were encountered in the lower part of the mid-region. This indicates that it could be beneficial to divide the working range into more operating regions or to change the partitioning of the operating regions. From the expressions for K_n and T_o, and also the obtained controller parameters, it can be seen that most of the process variation take place at lower K_La values. This suggests that the partitioning of the operating regions should be denser in the lower part of the working range. One could also improve the robustness by tuning the controllers in the lower part of each region.

Disturbances caused by anti-foam addition were well handled but did take considerable time to eliminate completely. At lower stirrer speeds, the excitation from sporadic disturbances, possibly due to air bubbles adhering to the sensor, seemed important.

5. Conclusions

This report has treated dissolved oxygen control in bioreactors when the stirrer speed is used as control input. In batch and fed-batch cultivations, the process characteristics may vary significantly and many authors have reported tuning problems when using controllers with fixed parameters. From a model of the process, it can be seen that the variations in the oxygen dynamics are due to changes in the volumetric oxygen transfer coefficient K_La.

If the performance needs are moderate, a PID controller with fixed parameters can be used. Maximum robustness against process variations is obtained if the controller is tuned for the lowest K_La-values that are likely to occur. This gives a stable closed-loop system under all operating conditions but the response will be sluggish at operating points with high K_La-values. When the performance demands are higher, gain-scheduling can be used to obtain similar performance at all operating points. The controller parameters should then depend on K_La. As K_La is affected by the stirrer speed, a simpler approach is to use the stirrer speed as the scheduling variable. In fed-batch and continuous cultivations, the load or the oxygen consumption is determined by the substrate feed rate. Feed-forward from the feed rate could then improve the overall control behavior.

Experiments with dissolved oxygen control in a laboratory bioreactor were performed during cultivations of *E. coli* bacteria. A PID controller combined with gain scheduling from the stirrer speed was tested. The controller was implemented using standard modules in an industrial control system. The approach was found to work well, yet some tuning problems were detected. This suggested that the partitioning of the operating regions should be made differently or that more operating regions may be useful. To improve the robustness further, the tuning should be made in the lower part of each interval. During the experiments anti-foam additions were made. The resulting disturbances were well handled but effects from the anti-foam could be observed for quite some time after the additions. It was also noted that sporadic disturbances, and their influence on the control, was more significant at low stirrer speeds.

Acknowledgments

The authors thank Lennart Andersson, Mikael Johansson and Tore Hägglund at the Department of Automatic Control, Lund Institute of Technology, for comments and suggestions on the manuscript. Thanks also to Anita Tocaj and Eva NordbergKarlsson at the Department of Biotechnology, Lund University, for invaluable help during the experiments.

This work was sponsored by Pharmacia & Upjohn which is gratefully acknowledged.

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