NORTH CAROLINA STATE UNIVERSITY

DEPARTMENT OF CHEMICAL AND BIOMOLECULAR ENGINEERING
CHEMICAL AND BIOPROCESS CONTROL:

"BTEC GROUP PROJECT: DISSOLVED OXYGEN IN A BIOREACTOR"

DR. GREG REEVES

APRIL 28, 2019

OMAR ALAM
CHRISTOPHER ATACK
TOLUWALASHE OMOTUNDE

INTRODUCTION

In this problem, cell cultures were spawned in different dissolved oxygen conditions. The end goal was to create a system that would have feedback control to maintain the dissolved oxygen level as close to the 50% of the air-based saturation concentration as possible.

A pure oxygen flow and air flow were mixed to provide the constant 25 SLPM flow feed to the tank. The manipulated variable in the system is the flow rate of the pure oxygen flow, controlled by an actuator that is responding to a controller. The controlled variable is the concentration of dissolved oxygen in the tank, measured by a sensor.

An oxygen mass balance will show the dynamics of exponential cell growth combined into the dynamics of mass transfer between actual dissolved oxygen and dissolved oxygen at saturation. Normalizing the dissolved oxygen concentrations to the saturation concentration of dissolved oxygen allows one to easily use the data given and to recognize using Henry's Law. By also relating the pure oxygen flow rate to oxygen gas mole percent, these adjustments are key in relating the manipulated variable of oxygen flow to the control variable of dissolved oxygen concentration. In this analysis, multiple constants needed to be evaluated, such as the mass transfer coefficient $k_l a$, the stoichiometric coefficient K_{O2}, maximum growth rate max, and initial cell dose xo.

The main goal is to maximize and sustain the cell growth as much as dissolved oxygen can affect it. The main concern is that not enough dissolved oxygen will prevent growth to the point that it is the limiting factor in its growth rather than food. But when oxygen concentration surpasses the air saturation level, reactive oxygen species (ROS), such as superoxide (O_2^-) and hydrogen peroxide (H_2O_2), accumulate as byproducts of aerobic metabolism. These are the main causes of cellular oxidative stress. However, the impact of dissolved oxygen concentration on *E. coli* processes for production of recombinant proteins is uncertain, and also varies depending on the specific strain.[1] Overall, it is more important to have good process control and output dynamics as this will allow the operator to test different set points to fit the situation.

RESULT AND DISCUSSION

CALCULATION OF UNKNOWN PARAMETERS

The DO equation (1) is first normalized by dividing by C^* to give equation (4), this is done because the data for the DO has been normalized to C^* and C^* can be gotten using equation (2). In order to find the DO, the unknowns in equation (1) must be calculated.

To find $k_l a$, the data from the sterile run in the excel sheet is used and the dynamics of the actuator is considered ignoring that of the sensor. Since this is a sterile run and there are no cells, x becomes 0 which simplifies equation (4). Equations (4) and (5) are then put in deviation variable format, Laplace transformed and inverted back to time domain to give $y_s(t)$ in equation (6). Using excel solver, $k_l a$ can be calculated by fitting $y_s(t)$ to the sterile run data.

To find μ_{max} and x_o, the cell density equation (7) is Laplace transformed and inverted back to time domain to yield x(t) in equation (8). The log of equation (8) is then taken to yield equation (9) which is in form of the equation of a straight line where the slope is μ_{max} and the intercept is

x_o. The equation is then fit to the first inoculated run looking only at time points prior to 180 minutes because it becomes non-linear after this time.
To find K_{O2}, equation (4) and (7) are Laplace transformed and inverted back into the time domain to yield equation (10) with the input being "u" and output "y(t)". $K_{O2}{}'$ is then calculated using excel solver to fit equation (10) to the data from both the 3 SLPM and 6SLPM innoculated runs and calculating the average. Nonlinearity arises after 90 minutes so only time points prior to 90 minutes are used in calculation. K_{O2} can now be calculated using equation (12).

To find H, Van't Hoff equation (11) is used and the parameters are gotten from NIST for oxygen at 1atm and 37^oC.

STABILITY CALCULATIONS FOR CONTROLLED RUNS

The stability calculations are done by getting the roots of the characteristic equation (20). The characteristic equation is gotten by multiplying the actuator, process, sensor and controller transfer functions. The resulting expression is then added to 1 and set to zero to give equation (20). For a sensor with no deadtime, equation (15) is used and when there's a 10seconds deadtime in the sensor equation (19) is used. Equation (20) is then expanded further using parameters from **TABLE 1** to get characteristic equations (21) for no deadtime and (22) for 10seconds deadtime. The corresponding K_c values for both controlled runs 1 and 2 gotten from **TABLE 1** are then plugged in equation (21) and (22) and the poles are gotten using Matlab.

For stability to occur, all the roots of the characteristic equation must be negative real roots. From **Appendix 1**, it is seen that one real root and two complex roots are obtained as poles of the characteristic equation for the runs without deadtime while two real roots and two complex roots are obtained from the runs with deadtime. The presence of complex roots in the characteristic equation results in instabilities in the dissolved oxygen concentration which makes it go above and below the setpoint.

STABILITY ANALYSIS

For stability to occur the oscillations gotten for the dissolved oxygen content must die out. This is not the case for both controlled runs so the lag is observed by plotting the controller and process output on the same graph. The lag is gotten from **Figure 1** and **2** using equation (23) for both controlled runs. If the Lag is closer to 0 compared to a quarter period, K_c is too high and if it is closer to a quarter period, τ_I is too small.

Controlled run 1 had a lag of 1.52 minutes and a quarter period of 0.5 minutes while controlled run 2 had a lag of 1.07 minutes and a quarter period of 0.38 minutes. This result indicates that the integration time (τ_I) is too small and increasing it will lead to stability in the system.

CONCLUSION

All in all, the process characteristics and dynamics were analyzed and understood. The unknown constants were calculated using the trials and experimental data. A simple feedback loop was projected, where the manipulated variable was the pure oxygen flow rate, essentially the mole fraction of oxygen in the feed flow, and the controlled variable was the dissolved oxygen concentration, revolving around a 50% saturation concentration set point.

Process control was important in this system, as the dissolved oxygen concentration depends on the exponential growth of the cells as well as being a first order differential equation, and so its dynamics are not understood at first glance. Incorporating dead time and the multiple transfer functions in the process allowed for the calculation of the roots of the overall transfer function. Understanding the roots allowed us to use the two controller variables Kc and I, to change the dynamics to those which will create a stable process. In the context of this data, the most obvious answer is to increase I significantly while keeping Kc constant. A repeat control run should be performed to understand how the process responds to the controller outputs, which adjustments to Kc and τ_I accordingly.

REFERENCES

1. Baez, A., & Shiloach, J. (2014). Effect of elevated oxygen concentration on bacteria, yeasts, and cells propagated for production of biological compounds. *Microbial cell factories*, *13*, 181. doi:10.1186/s12934-014-0181-5

TABLES

K_a	0.0316$SLPM^{-1}$
K_p	4.7619
K_c $(1st\ Run)$	22.1519SLPM
K_c $(2nd\ Run)$	44.3038SLPM
τ_p	0.132275
τ_s	0.16667min
τ_I	1.6667min
θ	0.16667min
u_{air}	0.21
PB $(1st\ Run)$	30%
PB $(2nd\ Run)$	15%

Table 1: Known Parameters

APPENDIX 1

UNKNOWN PARAMETERS

PARAMETERS	VALUES
$k_l a$	$7.56\ min^{-1}$
K_{O2}	$0.15197\ molL^{-1}OD^{-1}$
μ_{max}	$0.01875\ min^{-1}$
x_0	$0.1941\ OD$
H	$9.6238 \times 10^{-4} molL^{-1}$

ROOTS OF CHARACTERISTIC EQUATION
NO DEADTIME

POLES	CONTROLLED 1ST RUN	CONTROLLED 2ND RUN
S_1	-0.4767	-0.5323
S_2	-6.5416 + 12.1461i	-6.5136 + 17.2739i
S_3	-6.5416 – 12.1461i	-6.5136 - 17.2739i

WITH 10 SECONDS DEADTIME

POLES	CONTROLLED 1ST RUN	CONTROLLED 2ND RUN
S_1	-26.5716	-32.2539
S_2	-0.4846	-0.5376
S_3	0.7493 + 9.1629i	3.6170 + 10.6040i
S_4	0.7493 - 9.1629i	3.6170 - 10.6040i

EQUATIONS

1. $\frac{dC}{dt} = k_l a(C^*(t) - C) - K_{O2}\mu_{max}x$
2. $C^* = u\mathrm{H}$
3. $C = u_{air}H$
4. $\frac{dy}{dt} = k_l a\left(\frac{u}{u_{air}} - y\right) - K'_{O2}\mu_{max}x$
5. $\frac{dy_s}{dt} = \frac{1}{\tau_s}(y - y_s)$
6. $y_s(t) = \frac{0.0948}{0.21} + \frac{0.948k_l a}{0.21(1-10k_l a)}e^{\frac{-4.17}{10}} + \frac{0.0948}{0.21(10k_l a-1)}e^{-4.17k_l a} + \bar{y}$
7. $\frac{dx}{dt} = \mu_{max}x$
8. $x(t) = x_0 e^{\mu_{max}t}$
9. $\ln\big(x(t)\big) = \ln(x_o) + \mu_{max}t$
10. $y(t) = \frac{U(t)}{0.21}(1 - e^{-k_l at}) - \frac{K'_{O2}\mu_{max}x_0}{k_l a + \mu_{max}}(e^{\mu_{max}t} - e^{-k_l at}) + \bar{y}$
11. $H(T) = H_{25^oC}\exp\left(\frac{d(\ln(H))}{d(1/T)}\left(\frac{1}{T} - \frac{1}{25^oC}\right)\right)$
12. $K_{O2} = \frac{K_{O2'}}{uH}$
13. $K_c = \frac{100\%\Delta C}{PB\Delta y_s}$
14. $G_a = K_a = \frac{U(s)}{F_{spec}(s)} = \frac{1-u_{air}}{\dot{V}}$
15. $G_{s1} = \frac{Y_S(S)}{Y(s)} = \frac{1}{\tau_s s+1}$
16. $G_c = \frac{F_{spec}(s)}{E(s)} = K_c(1 + \frac{1}{\tau_I s})$
17. $G_p = \frac{Y(s)}{U(s)} = \frac{K_p}{\tau_p s+1}$
18. $\frac{dy_s}{dt} = \frac{1}{\tau_s}(y(t - \theta) - y_s)$

19. $G_{s2} = \frac{Y_S(S)}{Y(s)} = \frac{e^{-\theta_S s}}{\tau_S s+1}$

20. $G_a G_p G_c G_s + 1 = 0$

21. $\tau_s \tau_I \tau_p s^3 + \left(\tau_p \tau_I + \tau_s \tau_I\right) s^2 + \left(1 + K_a K_p K_c\right) \tau_I s + K_a K_p K_c = 0$

22. $\tau_s \tau_I \tau_p \theta_s s^4 + \left(2\tau_p \tau_I s + \tau_p \tau_I \theta_s + I \tau_s \theta_s\right) s^3 + \left(2K_a K_p K_c \tau_I - K_a K_p K_c \theta_s + 2\tau_I\right) s + \left(2\tau_I \tau_p + 2\tau_I \tau_s + \tau_I \theta_s - K_a K_p K_c \tau_I \theta_s\right) s^2 + 2K_a K_p K_c = 0$

23. $Lag = C_{trough} - Y_{peak}$

FIGURES

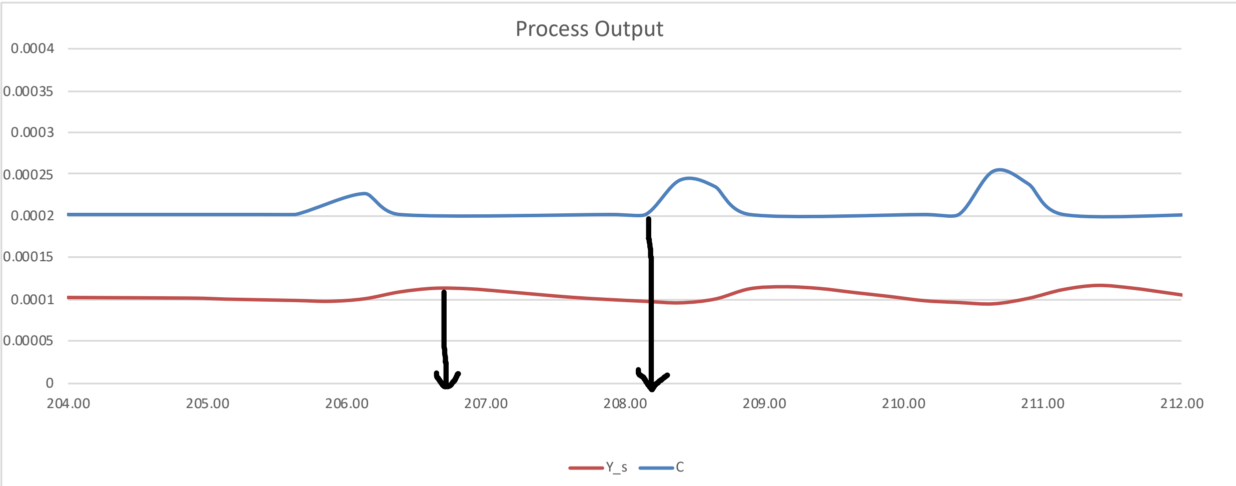

Figure 1: Controlled Run 1

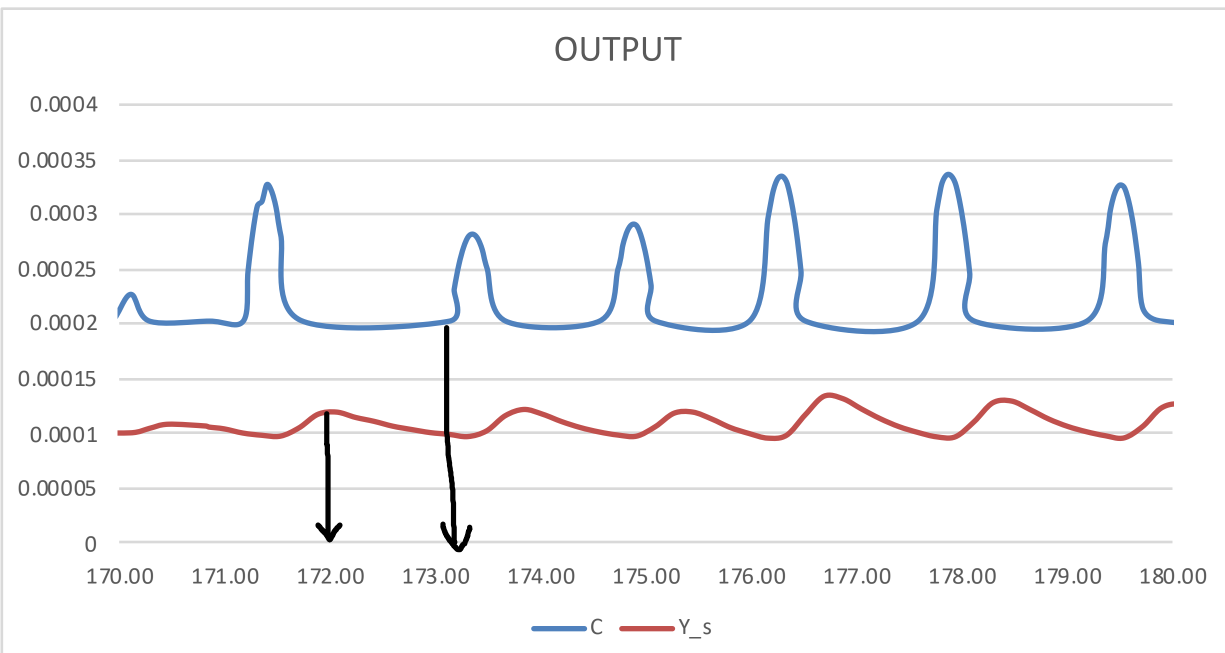

Figure 2: Controlled Run 2