ENHANCED MANAGEMENT OF DISSOLVED OXYGEN IN BIOREACTORS THROUGH KLA GRADIENT ASCENT TECHNIQUE

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ABSTRACT

The volumetric mass transfer coefficient (k_La) governs oxygen transfer from gas to liquid, influencing bioprocess productivity and stability. This study explores optimizing dissolved oxygen (DO) control in stirred-tank bioreactors by adjusting airflow rate (Q_G) and agitation speed (N), which are critical for biological cultures' optimal growth and metabolism. By employing experimental data from key control points, spline interpolation provides a precise modeling approach to determining optimal control parameters. The gradient ascent method is utilized to identify the maximum oxygen transfer rate, integral for precise DO management in the reactor. Although spline interpolation is non-global, it effectively delineates the control landscape, facilitating accurate numerical derivative approximations and the formulation of an optimal control path. Additionally, the study incorporates a Proportional-Integral-Derivative (PID) controller, which dynamically adjusts aeration and rotation based on real-time feedback, maintaining oxygen supply in equilibrium with biological demand. This methodology ensures that bioreactor operations are finely tuned to sustain desired k_La levels, enhancing overall efficiency and productivity.

Keywords: Static gassing-out. PID controller. Stirred-tank. Oxygen transfer rate. Python.

1 INTRODUCTION

The volumetric mass transfer coefficient (k_La) is a crucial parameter in the operation of bioreactors, playing a pivotal role in the management of dissolved oxygen levels, which are vital for the optimal growth and metabolism of biological cultures[1]. The k_La, defined by the Equation 1, sets the oxygen transfer rate (OTR) from the gas to the liquid phase, making its precise control fundamental in biotechnological applications, where k_L represents the mass transfer coefficient, a denotes the interfacial area, C is the oxygen concentration at a specific time t, and C^* is the equilibrium oxygen concentration.

$$\text{OTR} = dC/dt = k_La(C^* - C) \quad (1)$$

However, maintaining optimal k_La and dissolved oxygen levels presents significant challenges, particularly in the context of bioreactor design and operation. In aerobic cultivations, the level of dissolved oxygen (OD) is typically regulated to meet specific metabolic requirements by setting it as a fixed target. A cascade control system is often employed to adjust the bioreactor's agitation (rotation) and aeration rates in response to variations in oxygen uptake rate (OUR). These challenges are compounded in various bioreactor configurations, where the control strategies may include cascading control mechanisms - beginning with agitation followed by aeration adjustments. This lack of coordination may result in excessive shear stress and energy inefficiencies, ultimately impairing growth and productivity due to the system's adverse reactions to non-ideal control conditions. The objective of this study is to delineate a path of optimal control by selecting the best combinations of rotation speed (N) and aeration rate (Q_G) that maximize the control response to k_La. This involves understanding and predicting how changes in these variables influence k_La, thereby facilitating enhanced control strategies.

2 MATERIAL & METHODS

The k_La was determined using the static gassing-out method[1] under controlled conditions. Measurements were conducted at 25°C for distilled water with a viscosity of 0.87 mPa.s and for a 30% glycerol (v/v) solution with a viscosity of 3.23 mPa.s. These experiments were carried out in a 1-liter bioreactor equipped with two 3 cm diameter Rushton impellers spaced 3 cm apart. Below the lower impeller, a sparger with 2 µm pore diameter was installed. Dissolved oxygen (DO) concentration and temperature were monitored using Oxymax COS22D probe (Endress Hauser, Switzerland). Air and nitrogen flows were precisely measured and regulated using FMA-A2407 mass flow meters (Omega Engineering, USA).

In this study, cubic spline interpolation was used to model the relationship between control variables and k_La within the data's convex hull, ensuring interpolations remained within feasible boundaries. To achieve this, the Clough-Tocher scheme was employed by using the *scipy* package[2] in Python, ensuring smooth transitions without abrupt changes in curvature.

3 RESULTS & DISCUSSION

Managing dissolved oxygen (DO) in stirred-tank bioreactors begins by defining the control range for airflow rate (Q_G) and agitation speed (N). Within this framework, this study outlines a five-step methodology to optimize DO control in the bioreactor: k_La data

acquisition, $k_L a$ data interpolation, establishment of a gradient ascent path, formulation of the control law and estimation of optimal combinations of control parameters Q_G and N.

The $k_L a$ data were determined at critical points within the extremes of the control range and at a central point (corresponding to 200, 500 and 800 rpm and 0.3, 0.75 and 1.2 vvm). Given the physical expectation that $k_L a$ should increase with enhancements in both Q_G and N, it was imperative to accurately estimate the surface topology defined by these data points. Unlike polynomial or other analytic regression techniques, spline interpolation does not yield a single formula encompassing the entire domain. Instead, it employs piecewise cubic polynomials, with each segment between data points being independently defined. Consequently, while this method excels in interpolation within the known data range, extrapolating beyond this range can produce unreliable outcomes. However, since the $k_L a$ experimental data points strictly correspond to the operational control range, extrapolation is not a concern in our analysis.

Upon constructing the interpolated grid from the $k_L a$ data points, it becomes possible to delineate the optimal control path that should be followed to maximize the response over $k_L a$. This optimization is facilitated through the application of the gradient ascent method, a numerical technique designed to locate the maximum of a function. In this context, the function f represents the interpolated $k_L a$ values over a grid defined by Q_G and N, as delineated in Equation 2. The gradient of the function f, denoted as ∇f, is a vector composed of partial derivatives. It indicates the direction of the steepest ascent in the function's value. Mathematically, this vector is expressed as shown in Equation 3.

$$f(Q_G, N) = k_L a \tag{2}$$

$$\nabla f(Q_G, N) = \left(\frac{\partial f}{\partial Q_G}, \frac{\partial f}{\partial N}\right) \tag{3}$$

In practical applications, especially when using piecewise spline interpolation, despite the smoothness and continuity, there is not a single global analytical expression for f. Consequently, derivatives of f must often be approximated numerically rather than derived from a unified analytical formula. As f can be represented on a discrete grid, finite differences might be used, as delineated by Equation 4 and 5, where h is a small step in the respective variable.

$$\frac{\partial f}{\partial Q_G} \approx f\left(Q_G + h_{Q_G}, N\right) - f\left(Q_G - h_{Q_G}, N\right)/\left(2h_{Q_G}\right) \tag{4}$$

$$\frac{\partial f}{\partial N} \approx f(\mathrm{Q_G}, N + h_N) - f(\mathrm{Q_G}, N - h_N)/(2h_N) \tag{5}$$

Starting from an initial point (Q_{G_0}, N_0) – minimal control point – the function is maximized by iteratively updating the values of Q_G and N using Equation 6 and 7 for each component, where α is the learning rate or step size, which controls the magnitude of parameter adjustments in each iteration. This step size was carefully tuned to ensure convergence without overshooting by choosing between two calculated values (a dynamically scaled step size and a minimum step size, $\alpha_0 = 0.01\alpha$), given by the Equation 8, where the dynamically scaled step size is calculated by multiplying the Euclidean norm of the gradient by a scaling factor defined as $\alpha^* = 0.2\alpha$. This ensures that each update step is proportional to the current gradient's strength, allowing faster convergence in steep areas and careful stepping in flat regions. The iteration continues until a stopping criterion is met, typically set when the gradient ∇f becomes very small, indicating a local maximum or plateau has been reached. Consequently, the path traced by (Q_{G_n}, N_n) over iterations effectively forms the ascent path in the function's landscape.

$$Q_{G_{n+1}} = Q_{G_n} + \alpha \cdot \left.\frac{\partial f}{\partial Q_G}\right|_{(Q_{G_n}, N_n)} \tag{6}$$

$$N_{n+1} = N_n + \alpha \cdot \left.\frac{\partial f}{\partial N}\right|_{(\mathrm{Q_{G_n}}, N_n)} \tag{7}$$

$$\alpha = \max_n\left(\alpha^* \cdot \left\|\nabla f\left(Q_{G_n}, N_n\right)\right\|, \alpha_0\right) \tag{8}$$

By dynamically adjusting the $f(Q_G, N)$ in response to changes in OUR, the control system leverages the established path to ensure that OD levels are maintained within optimal bounds for the bioprocess. Adjustments are made based on real-time feedback to modify the oxygen transfer rate (OTR), thereby increasing, decreasing, or stabilizing the dissolved oxygen (OD). To maintain OD at a constant level, it is crucial to align OTR with OUR, ensuring equilibrium between oxygen supply and demand. This balance is achieved using a Proportional-Integral-Derivative (PID) controller, which adjusts $k_L a$ (and indirectly Q_G and N) based on deviations of the actual oxygen concentration C from a predefined target C_{target}. The PID control law is defined in Equation 8, while the control error at time t, $e(t)$, is described in Equation 9, where K_P, K_I, and K_D are the proportional, integral, and derivative gains, respectively.

$$\Delta k_L a = \Delta f = K_P e(t) + K_I \int e(t)dt + K_D \frac{d}{dt} e(t) \quad (8)$$

$$e(t) = C_{target} - C(t) \quad (9)$$

The output of the control law determines the $k_L a_{target}$, which guides the selection of Q_G and N settings along the optimal path. To minimize computational demands, a simple linear interpolation is utilized, allowing for fewer data points without sacrificing precision. This interpolation method is applied when the $k_L a_{target}$ lies between two consecutive path points, $k_L a_n$ and $k_L a_{n+1}$. The corresponding Q_G and N values are then calculated using the Equations 10 and 11.

$$Q_{G\,target} = Q_n + \frac{(Q_{G_{n+1}} - Q_{G_n})}{(k_L a_{n+1} - k_L a_n)} \cdot (k_L a_{target} - k_L a_n) \quad (10)$$

$$N_{target} = N_n + \frac{(N_{n+1} - N_n)}{(k_L a_{n+1} - k_L a_n)} \cdot (k_L a_{target} - k_L a_n) \quad (11)$$

The interpolated values, $Q_{G\,target}$ and N_{target}, are subsequently used to adjust the control parameters for the actuators – specifically, the flow meter and motor. This alignment ensures that the bioreactor operations are finely tuned to meet the desired $k_L a_{target}$, while also minimizing the computational load by reducing the necessity to store and process a large number of data points. The Figure 1 presents the interpolated grid and ascend control path generated for distilled water and 30% v/v glycerol solution.

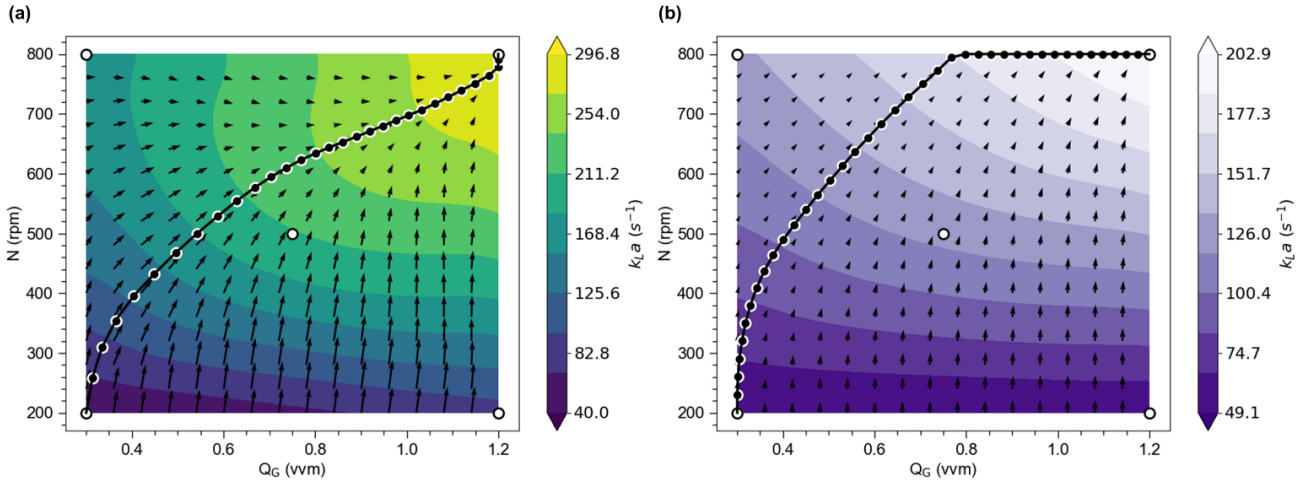

Figure 1 . Spline-interpolated response surface for $k_L a$ based on variations in Q_G and N for **(a)** distilled water and **(b)** 30% v/v glycerol solution. White dots indicate experimental data points, while black arrows show the gradient direction. Black dots denote discrete path points derived through the gradient ascent method, and the black line represents the interpolated path used to select the target values $Q_{G\,target}$ and N_{target} .

4 CONCLUSION

This study has successfully developed a control methodology utilizing interpolation techniques to regulate oxygen levels within a precise range. From experimental $k_L a$ data collected at both the centroid and extremities of control settings, this approach dynamically corrects deviations from targeted oxygen concentrations through tailored adjustments in airflow rate and agitation speed. These modifications enhance the control authority over $k_L a$ variations defined by the PID controller, allowing for the optimal selection of control paths under varying operational conditions. Consequently, this strategy not only enables an ideal environment for microbial growth and productivity but also improves energy efficiency, thereby rendering the bioprocess more sustainable and cost-effective.

REFERENCES

[1] CERRI, M. O., ESPERANÇA, M. N., BADINO, A. C., RIBEIRO, M. P. A. 2016. A new approach for kLa determination by gassing-out method in pneumatic bioreactors. J. Chem. Technol. Biotechnol. 91 (1). 3061-3069.

[2] VIRTANEN, P., GOMMERS, R., OLIPHANT, T. E. et al. 2020. SciPy 1.0: fundamental algorithms for scientific computing in Python. Nat. Methods. 17 (1). 261-272.2

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