A Real-time Adaptive Oxygen Transfer Rate Estimator for Metabolism Tracking in *Escherichia coli* Cultures*

Li Wang, Matthew E. Pepper, Ajay Padmakumar, Timothy C. Burg, Sarah W. Harcum, and Richard E. Groff

***Abstract*— Oxygen transfer rate (OTR) is the most significant signal for aerobic bioprocess control, since most microbic metabolic activity relies on oxygen consumption. However, accurate estimation of OTR is challenging due to the difficulty of determining uncertain oxygen transfer parameters and system dynamics. This paper presents an adaptive estimator, which incorporates exhaust gas, stir speed and dissolved oxygen measurements, to predict the real-time OTR. The design of this estimator takes into account the headspace dilution effect, off-gas sensor dynamics and uncertain oxygen transfer parameters. Through simulation the estimated real-time OTR is shown to accurately track quick changes of oxygen demand in the culture. Thus, it can be applied to a variety of controls and estimation purposes, such as determining when the culture is in oxidative or overflow metabolism.**

I. INTRODUCTION

Oxygen transfer rate (OTR) is the most significant measurable online signal for aerobic bioprocess control. It can be used to quantify different physiological states of the culture by determining the related oxygen consumption[1]. The shape of the OTR signal indicates rich information, such as substrate limitation, oxygen limitation, product inhibition and diauxic growth, about the metabolism of aerobic micro-oganisms[2].

The determination of real-time OTR is challenging, because it depends on the oxygen solubility, stir speed, cultivation media, temperature, pressure and other factors. Methods for determining OTR are categorized as oxygen transfer coefficient method or off-gas analysis method. For the oxygen transfer coefficient method, oxygen transfer capability is often characterized by a lumped parameter called the liquid-side volumetric mass-transfer coefficient k_La[3]. Much prior work has explored the experimental methods for determining k_La.

The oxygen transfer coefficient method can be classified as physical or chemical methods. Physical methods directly measures the oxygen concentration of the solution, while chemical methods measures the reaction rate of a homogeneous reaction. Most of these methods calculate the k_La when there are no cells or other uncertain organisms consuming the dissolved oxygen. However, k_La value will drift away from the pre-calibrated value when cells are added to the culture. Thus, the real-time OTR predicted from those predefined k_La values are inaccurate. OTR estimation for oxygen consuming system often assumes that OUR (oxygen uptake rate) and k_La remains constant for a short period of time[4]. This assumption makes it impossible to track quick changes in $k_La$online. This method is also not efficient when k_La needs to be determined regularly during the experiment.

The off-gas analysis method directly measures the amount of oxygen transferred into the solution with an off-gas analyzer. It provides a more reliable source of real-time OTR estimation. Recent advances in the sensor development offers low cost off-gas analyzer(e.g. BlueSens Gas Sensor GmbH, Herten, Germany) with comparable accuracy to the established benchmark standard mass spectrometer. The reduced cost makes it feasible to dedicate a sensor to each bioreactor to enable more sophisticated real-time estimation and control of bioprocesses. However, direct usage of off-gas sensor measurement is problematic, because the sensor measures a heavily filtered version of OTR. The dilution effect of headspace and sensor measurement dynamics exert significant lag on the OTR, filtering out quick changes in OTR. Previous work on off-gas analysis ignores filtering effects, because lag in the OTR signal is acceptable for slow monitoring applications. However, those effects need to be considered for more advanced real-time bioprocess estimation and control.

This paper reconstructs real-time OTR by modeling the dilution effect of the head space and the lag of the off-gas sensor. The prior unknown and time-vary parameter k_La is linearized by the stir speed. The parameters of the k_La linearization are adaptively updated using off-gas measurements. OTR estimate is then calculated from stir speed and dissolved oxygen level using the adaptive k_La parameters to account for the slow variations during the entire experiment. The organization of the rest of the paper is as follows. The

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mathematical model of the bioreactor oxygen transfer system is formulated in section II. Then, an adaptive estimator based on the oxygen transfer dynamics is presented in section III. A simulated fermentation experiment is performed to validate the estimator design in section IV. The characterization of the off-gas sensor is provided in V. The paper is concluded in section VI with a summary and discussion of future applications.

II. System Modelling

The real-time OTR estimator is based on a known oxygen transfer model with unknown parameters. The model will be discussed in this section. Let b_0, b_1, b_2, b_3 denote the mole percent oxygen of the gas inputting into the culture, the gas coming out of the culture, the gas in the head space of the bioreactor and the off-gas sensor reading respectively. Figure 1 illustrates the oxygen transfer model variables. Where V_1 is the volume of the culture, V_2 is the volume of the head space.

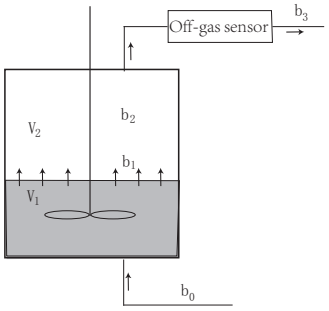


Fig. 1: Variables for oxygen transfer model

Theoretically, OTR can be calculated with the oxygen percentage difference between the gas entering and exiting the culture.

$$\mathrm{OTR} = \frac{M_f(b_0 - b_1)\rho_{o_2}}{V_1}, \tag{1}$$

where M_f is the mass flow rate, ρ_{o_2} is the oxygen density at $37C^o, 1\ atm$.

The off-gas sensor is used to measure the oxygen percentage coming out of the bioreactor. However, the sensor can not directly measure the off-gas percentage b_1. Instead, the sensor samples b_2 and its reading b_3 has a lag. The gas coming out of the culture first mixes with the gas in the head space of the bioreactor. This mixing process is modeled as a first order dilution process with time constant $\tau_1 = \frac{V_2}{M_f}$. At the same time, the sensor measurement dynamics can be characterized as a first order system with time constant τ_2, which is independent of M_f by the experiment discussed in section V.

$$\dot{b_2} = \frac{M_f}{V_2}(b_1 - b_2), \tag{2}$$

$$\dot{b_3} = \frac{1}{\tau_2}(b_2 - b_3) \tag{3}$$

The off-gas sensor reading b_3 can be viewed as a heavily filtered version of b_1. Directly deriving b_1 from b_3 will introduce noise to the signal, because it involves taking the second derivative of b_3. Let $\bar{\mathbf{x}} = [b_2, b_3]^T$, Equation (2) and (3) can be written as state space form:

$$\dot{\bar{\mathbf{x}}} = \begin{bmatrix} -\frac{M_f}{V_2} & 0 \\ \frac{1}{\tau_2} & -\frac{1}{\tau_2} \end{bmatrix} \bar{\mathbf{x}} + \begin{bmatrix} \frac{M_f}{V_2} \\ 0 \end{bmatrix} b_1 \tag{4}$$

Another way of real-time OTR determination is to consider the volumetric oxygen transfer coefficient k_La. k_La has a strong linear relationship with stir speed[6].

$$\mathrm{OTR} = k_La(C^* - C), \tag{5}$$

$$k_La = \alpha_0 + \alpha_1(N - N_0), \tag{6}$$

where k_La is linearized around a stir speed N_0. The relation between k_La and stir speed does not remain constant during the fermentation, due to the change of liquid composition and viscosity. Hence, the parameters α_0 and α_1 vary during the course of a fermentation experiment. The method presented here combines the off-gas sensor measurement with the stir speed and dissolved oxygen readings to estimate α_0 and α_1 adaptively. This approximation of k_La is timely unlike other methods, because of its dependance on online sensors.

From Equation (1), (5) and (6), b_1 can be written as:

$$b_1 = b_0 - \frac{V_1(C^* - C)}{M_f\rho_{o_2}}\alpha_0 - \frac{V_1(C^* - C)(N - N_0)}{M_f\rho_{o_2}}\alpha_1 \tag{7}$$

Combining (7) and (4), the oxygen transfer dynamics can be reformulated as observable canonical form:

$$\dot{\mathbf{x}} = A\mathbf{x} + B\mathbf{f}, \tag{8}$$

$$y = C\mathbf{x}, \tag{9}$$

$$A = \begin{bmatrix} -\frac{M_f}{V_2} - \frac{1}{\tau_2} & 1 \\ -\frac{M_f}{V_2\tau_2} & 0 \end{bmatrix}, B = \begin{bmatrix} 0 & 0 & 0 \\ \alpha_0 & \alpha_1 & b_0 \end{bmatrix},$$

$$C = [1\ 0], \mathbf{f} = \begin{bmatrix} f_0 \\ f_1 \\ f_2 \end{bmatrix} = \frac{1}{\tau_2} \begin{bmatrix} \frac{-V_1(C^* - C)}{V_2\rho_{o_2}} \\ \frac{-V_1(C^* - C)(N - N_0)}{V_2\rho_{o_2}} \\ \frac{M_f}{V_2} \end{bmatrix},$$

where the system output y is the off-gas sensor measurement, the system input $\mathbf{f}$ is a vector of functions of the dissolved oxygen level and stir speed signals.

III. OTR Estimator Design

If α_0 and α_1 are two slowly time varying unknown parameters, then an adaptive observer of the following form can be designed to achieve simultaneous state and parameter estimation[7].

$$\dot{\hat{\mathbf{x}}} = A\hat{\mathbf{x}} + \begin{bmatrix} 0 \\ \hat{\alpha}_0 \end{bmatrix} f_0 + \begin{bmatrix} 0 \\ \hat{\alpha}_1 \end{bmatrix} f_1 + \begin{bmatrix} 0 \\ b_0 \end{bmatrix} f_2 + p + q, \tag{10}$$

$$\hat{y} = C\hat{\mathbf{x}}, \tag{11}$$

where p and q are auxiliary variables compensating the parameter estimation errors. The estimation error dynamics can be derived by taking the difference between the estimator and the real system.

$$\dot{\mathbf{e}} = A\mathbf{e} + \phi f_0 + \psi f_1 + p + q, \quad (12)$$

$$\mathbf{e}_1 = C\mathbf{e}, \quad (13)$$

where $\mathbf{e} = \hat{\mathbf{x}} - \mathbf{x}$, $\mathbf{e}_1 = \hat{y} - y$, $\phi = [0, \hat{\alpha}_0 - \alpha_0]^T$, $\psi = [0, \hat{\alpha}_1 - \alpha_1]^T$, $p = [0, \ -4\dot{\phi}_1\omega_2 + \dot{\phi}_2\omega_2]^T$, and $q = [0, \ -4\dot{\psi}_1 v_2 + \dot{\psi}_2 v_2]^T$. $\omega(t) = G(s)f_0(t)$, $v(t) = G(s)f_1(t)$ and $G(s) = [\frac{s}{s+4}, \frac{1}{s+4}]^T$.

The adaptive law is chosen as $\dot{\phi} = -\gamma_0 \mathbf{e}_1 \omega$, $\dot{\psi} = -\gamma_1 \mathbf{e}_1 v$, where γ_0 and γ_1 are adaptive gains. The real-time OTR signal is derived with the adaptively updated parameters $\hat{\alpha}_0$ and $\hat{\alpha}_1$

$$\hat{\text{OTR}} = (\hat{\alpha}_0 + \hat{\alpha}_1(N - N_0))(C^* - C), \quad (14)$$

IV. Simulation Results

The tracking performance of the adaptive estimator was first evaluated with Matlab simulation. A model developed by Xu et al.[5] is adopted to simulate how the *E. coli* cells take in substrate and oxygen to produce biomass, while generating carbon dioxide and acetate. The model consists of the dynamic equations of biomass, substrate, acetate, oxygen and growth rate. It accurately emulates the metabolism of the cells.

E. coli has three different phases of metabolism, i.e. oxidative, overflow and metabolite consumption[5]. In the oxidative metabolism phase, the cells process glucose aerobically without producing harmful byproduct. When excessive amount of glucose exists in the environment, the cells go to overflow metabolism, processing glucose anaerobically while producing growth inhibitor(acetate). The metabolite consumption processes acetate when glucose supply becomes limited. The principle of growth maximizing controller is to maximize oxidative metabolism while avoiding the overflow phase. OUR is an excellent indicator for overflow because it plateaus when cells enter overflow phase.

The overall performance of the estimator in a 14.5 hours simulated *E. coli* fermentation is illustrated in fig. 3. OTRxu is generated by the Xu uptake model. The estimated OTRest gradually converges to OTRxu at the 4th hour and keeps the good tracking performance until the end of the fermentation experiment. During the fed-batch phase, the glucose feeding rate increases by every 1% discrete steps due to the resolution limitation of the pump. It is also shown that every step change of the glucose feeding rate also leads to a step change in OTRxu. The estimator tracks exactly the quick step changes in OTRxu, while OTRsen, i.e. value calculated directly from the off-gas sensor, has significant lag and fails to capture those quick changes.

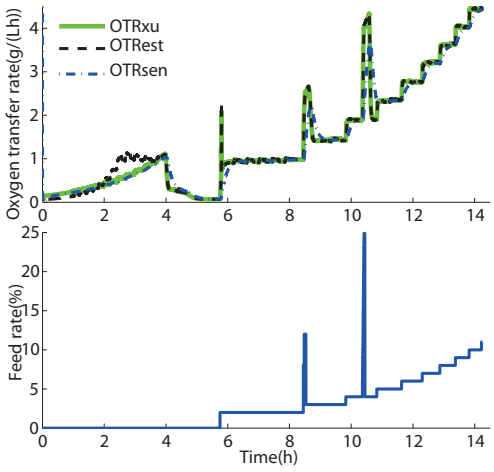
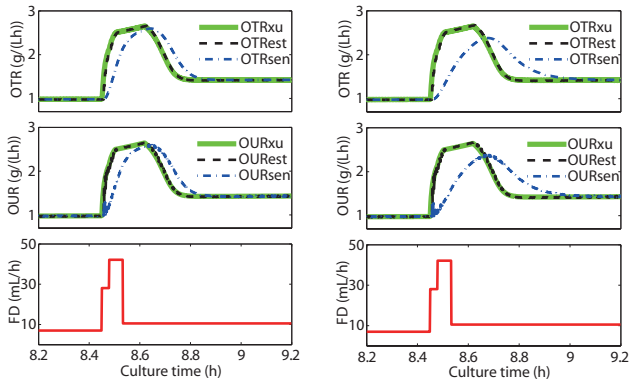


Fig. 2: Simulated OTR tracking: OTRxu is generated by Xu model, OTRest is the estimated OTR, OTRsen is the OTR calculated directly from the off-gas sensor

Two glucose feeding pulses(at t = 8.4h and t = 10.3h) are applied to the culture during the fed-batch phase. The estimator successfully compensates the lag in the system and accurately tracks the pulse in the OTRxu. As shown in Fig. 3a, OTRxu spikes immediately after the pulse and then plateaus, which indicates that the cells enter overflow metabolism. OTRest successfully tracks the sudden changes in OTRxu, while the OTRsen experiences significant lag and fails to capture the plateau region of OTRxu. OTRest is more accurate and timely as compared with other methods. Note that OUR directly characterizes the metabolism of the cells. However, $\text{OTR} \approx \text{OUR}$ in Fig. 3a.

The filtering effect of the head space will be more obvious when the head space is larger and mass flow rate is smaller. The time constant of the headspace dilution model is $\tau_1 = \frac{V_2}{M_f}$. When the mass flow rate M_f decreases from 3 lpm to 1 lpm, τ_1 will be three times larger, i.e. the lag in OTRxu signal will be more significant. As illustrated in figure 3b, OTRest still tracks the real OTR with high accuracy when $M_f = 1$ lpm, while OTRsen experiences significant lag. OTRsen completely missed the plateau region, which indicates the cells enter overflow metabolism. Fermentation under low mass flow rate is the common case for mammalian cells, which requires much less oxygen consumption than *E. coli* . The advantage of using OTRest rather than OTRsen will be more obvious for those low mass flow rate applications.

A more interesting potential application of the estimator is shown in Fig. 4. When a ramped pulse of glucose feed rate is executed, OTRxu gradually increases and reaches the maximum value. By comparing the real-time feeding profile and OTRest signal, the estimator



(a) Simulated pulse(M_f=3 lpm)(b) Simulated pulse(M_f=1 lpm)

Fig. 3: OTR estimation in simulated *E. coli* fermentation, OUR is calculated by $\mathrm{OUR} = \mathrm{OTR} - \dot{C}$. FD is the glucose feed rate during the fermentation.

can be used to identify the exact glucose feed rate(21 mL/h) at which the cells enter overflow metabolism. By performing the ramped glucose pulse periodically during the fermentation, the maximum feed rate can be identified and used for maximizing growth control.

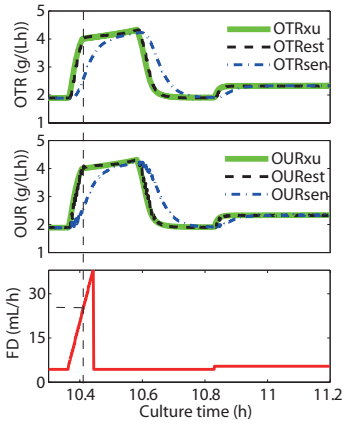


Fig. 4: Simulated ramped glucose pulse(M_f=3 lpm)

V. Sensor Characterization Experiment

In order to implement the adaptive OTR estimator on the system, the time constant τ_2 for the off-gas sensor must be identified. The BlueInOne off-gas analyzer was adopted for the fermentation experiment.

In the characterization experiment, the input gas b_0, was connected directly to the input of the BlueInOne sensor, bypassing the stirred-tank vessel; this allowed for measurement of only the sensor dynamics free from the gas transfer lag. As shown in Fig. 5, the composition and flow rate of the input gas was varied in the experiment. The time constant of the off-gas sensor $\tau_2 = 55s$, i.e., the time for the sensor to reach 63% of its final value, is independent of input mass flow rate in the operating range (1 lpm - 8 lpm).

This experiment validates that sensor measurement dynamics can be modeled as a first order system.

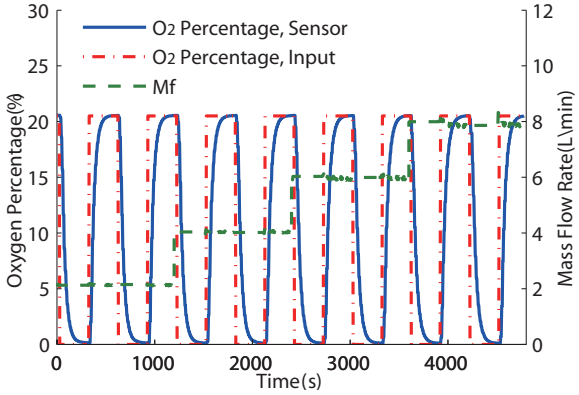


Fig. 5: Bluesens time constant identification under different input gas mass flow rate

VI. Conclusions

This paper presents an adaptive estimator to accurately track the real-time OTR of aerobic bioprocess. The estimator compensates the effect of head space dilution, sensor lag and time-varying oxygen transfer parameters. The performance of the estimator is demonstrated with simulated fermentation experiment. The application of the OTR estimator to determine overflow metabolism phase during the *E. coli* fermentation is elaborated. The estimator developed in this paper enables accurate tracking of the quick changes in OTR, thus providing timely indicator for culture states and metabolism changes. The OTR estimator will be an integral part in the development of maximizing controllers for oxidative metabolism. Future work will analyze estimator performance during *E. coli* fermentation experiment.

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