Simultaneous Determination of Oxygen Consumption Rate and Volumetric Oxygen Transfer Coefficient in Pneumatically Agitated Bioreactors

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A new approach is proposed for the simultaneous determination of the volumetric oxygen transfer coefficient (K_La) and the oxygen uptake rate (OUR) in bioreactors. The methodology is based on modifications of the classical dynamic gassing-in method and the steady-state mass balance method for obtaining the values K_La and OUR, respectively. A polarographic dissolved oxygen electrode is used to monitor the oxygen concentration during a switch of aeration gas composition. The flow rate of the gas phase is not altered so that the hydrodynamics of the bioreactor are not affected during the measurement. Data obtained with this method are compared with the classical methods to show that the proposed technique is robust and reproducible. At a 95% confidence level, the proposed method produced results that were statistically identical to data obtained with the traditional methods. The proposed technique was reproducible to within 4% of the mean value at a 95% confidence level. The proposed method was further proved by applying it to a plant-scale (17-L) batch culture of the microfungus *Aspergillus terreus* in a fluidized-bed reactor.

1. Introduction

Providing sufficient oxygen and removing carbon dioxide are important in the design and operation of aerobic bioreactors. Gas−liquid mass transfer in bioreactors is characterized in terms of the overall volumetric oxygen transfer coefficient (K_La) and the oxygen uptake rate (OUR). Accurate knowledge of the values of K_La and OUR is essential for design and operation. These variables are affected by many factors, including viscosity and surface tension of the broth; the concentration and morphology of the biomass; bioreactor hydrodynamics; and aeration rate. Prediction of K_La values in biological systems is difficult; hence, data measured in culture broths are necessary for various purposes.

Several methods are available for measuring K_La,[1] but few of these are applicable to measurements in biological systems during operation. The well-known dynamic method[2] is one of the most frequently used techniques for measuring K_La during culture. The method relies on measuring the dissolved oxygen concentration versus time profiles. In a first step, the gas flow that delivers oxygen to the bioreactor is stopped and the rate of oxygen depletion by microbial uptake is measured to determine the oxygen consumption term (OUR). Subsequently, the gas flow is restored to the initial operating value and the increase in concentration of dissolved oxygen with time is used to calculate the K_La. The dissolved oxygen is measured using sensing electrodes. The dynamic measurement requires interruption of air flow. This invariably affects the hydrodynamics of the bioreactor during the measurement. Furthermore, the dynamic method assumes that the oxygen consumption term is not affected by changes in the hydrodynamic regimen.

Because it interrupts gas flow, the dynamic method is not really suitable for pneumatically mixed bioreactors where the sparged gas stream is the sole source of mixing. Clearly, with the gas flow interrupted in a pneumatically agitated bioreactor, the fluid is no longer "well-mixed", violating an important assumption of the dynamic method. The present work demonstrates a modification of the dynamic method such that the flow rates of aeration gas are not altered during the measurements of K_La and OUR. The proposed method is broadly applicable to any biological system in which oxygen is transferred by aeration.

There is ample literature on various approaches for measuring K_La,[1−8] and some of the proposed methods have been specifically applied to industrial-scale fermentation processes. Existing techniques such as the start-up dynamic method and the dynamic pressure method[4−6] are not entirely suitable for pneumatically agitated bioreactors. Pressure changes are known to affect hydrodynamics of flow by altering the gas holdup and gas−liquid interfacial areas.[9] A method involving variation of the composition of aeration gas has been used for determining K_La, but only in nonbiological systems that did not involve oxygen consumption.[6]

The modified dynamic method presented in this work for simultaneous measurement of K_La and OUR is applied to two different systems used for culturing the microfungus *Aspergillus terreus*. The proposed method overcomes the problems associated with the classical dynamic method, by not requiring any change to the flow rate of the aeration gas. Changes in composition of the aeration gas are used exclusively, and there is no disturbance of the bioreactor hydrodynamics during the measurement.

2. Theoretical Background

In a gas−liquid system, the mass balance of oxygen in the liquid phase is as follows:

$$dC_L/dt = K_La(C^* - C_L) - xq_{O_2} \tag{1}$$

In eq 1, dC_L/dt is the accumulation of oxygen in the liquid phase, $K_La(C^* - C_L)$ represents the oxygen transfer rate from the gas to the liquid phase, q_{O_2} is the specific oxygen consumption rate, and x is the biomass concentration, with the product $-xq_{O_2}$ being the oxygen uptake rate (or OUR).

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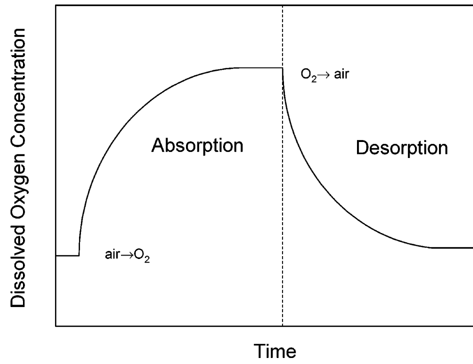

Figure 1. Absorption−desorption cycle for the modified dynamic method.

The classic formulation of the dynamic method for the determination of K_La relies on a two-step desorption−absorption cycle. During the net consumption or desorption step, the oxygen transfer rate is nil as there is no flow of aerating gas, i.e., $K_La(C^* - C_L) = 0$. For this situation, eq 1 can be integrated to

$$C_L = -xq_{O_2}t \tag{2}$$

Thus, $-xq_{O_2}$ (OUR) can be obtained as the slope of a plot of C_L versus time. The specific oxygen consumption q_{O_2} is then easily calculated using the measured x value.

During the aeration step following the interruption of gas flow, oxygen is absorbed into the broth. Both mass transfer and oxygen consumption occur together. The C_L versus time curve is now described by the following equation:

$$C_L = -\frac{1}{K_La}\left\{\left(\frac{dC_L}{dt}\right) + xq_{O_2}\right\} + C^* \tag{3}$$

Equation 3 is a rearranged form of eq 1. For a given biomass concentration and known q_{O_2}, K_La is obtained as the slope of a plot of C_L versus $\{(dC_L/dt) + xq_{O_2}\}$. The y-intercept of this plot provides the value of C^*, the equilibrium saturation concentration of dissolved oxygen in the broth.

The drawback of the dynamic method in its original formulation is that the calculation relies on simplifying eq 1 to eq 2 by stopping the aeration flow and eliminating absorption of oxygen. The modified method proposed in the present work does not require stopping of the aeration flow. Instead, a step change in composition of the aeration gas is imposed without altering its flow rate. The consequent transition between two steady states of dissolved oxygen concentration is mathematically analyzed to obtain K_La and OUR. The absorption−desorption cycle used is shown in Figure 1. Starting from a low oxygen concentration, C_{L0} at t_0, a change in the gas-stream composition promotes oxygen absorption with a driving force of $(C_1^* - C_L)$. The dissolved oxygen concentration is monitored until some time t_1 when a new oxygen concentration C_{L1} has been attained. Then the cycle is completed by changing the gas-stream composition to cause desorption with a driving force of $(C_L - C_2^*)$ over a time period t_2 when the oxygen concentration in the liquid has become C_{L2}. With the absorption−desorption strategy used, eq 1 can be applied to both parts of the cycle with the following initial conditions:

Absorption:

$$t = 0 \quad C^* = C_0^* \quad C_L = C_{L0}; \quad t = t_1 \quad C_L = C_{L1}$$

Desorption:

$$t = t_1 \quad C^* = C_1^* \quad C_L = C_{L1}; \quad t = t_2 \quad C_L = C_{L2}$$

K_La and q_{O_2} can be obtained by fitting the two equations that result from integrating eq 1 with the two sets of initial conditions noted above to the experimental data of the absorption−desorption cycles.

The process of obtaining K_La becomes easier if eq 1 is first integrated analytically. Assuming that xq_{O_2}, C^*, and K_La remain constant, a new variable α, defined by eq 4, is also a constant:

$$\alpha = K_LaC^* - xq_{O_2} \tag{4}$$

Using this definition in eq 1, the following equation is obtained:

$$dC_L/dt = \alpha - K_LaC_L \tag{5}$$

Equation 5 can be integrated as follows:

$$\int_{C_{L0}}^{C_L} dC_L/(\alpha - K_LaC_L) = \int_0^t dt \tag{6}$$

leading to

$$\left(\frac{\alpha - K_LaC_L}{\alpha - K_LaC_{L0}}\right) = e^{-K_Lat} \tag{7}$$

After substituting α (eq 4) and imposing the initial conditions for the two cycles, the above equation can be rearranged to the following system representing the two steps of the method:

$$C_L = \left(C_0^* - \frac{xq_{O_2}}{K_La}\right) + \left(C_{L0} - C_0^* + \frac{xq_{O_2}}{K_La}\right)e^{-K_Lat} \tag{8}$$

$$C_L = \left(C_1^* - \frac{xq_{O_2}}{K_La}\right) + \left(C_{L1} - C_1^* + \frac{xq_{O_2}}{K_La}\right)e^{-K_Lat} \tag{9}$$

Equations 8 and 9 describe the oxygen concentration versus time curves from some initial starting concentration (C_{L0} or C_{L1}, measured at the start of absorption and desorption steps, respectively) to the instance where the composition of the gas stream is changed given an equilibrium concentration in the liquid phase of C_0^* or C_1^*.

Equations 8 and 9 are of the form $C_L = y_0 + a\ e^{-bt}$, where $y_0 = (C^* - xq_{O_2}/K_La)$, $a = (C_{L0} - C^* + xq_{O_2}/K_La)$ and $b = K_La$. The parameters y_0, a, and b can be obtained by a nonlinear regression of the measured C_L versus t data. Since C_{L0} and x (the biomass concentration) are known, C^*, q_{O_2}, and K_La can be readily calculated. Separate values are obtained for absorption and desorption steps. The equations used in calculating q_{O_2} and C^* are as follows:

$$q_{O_2} = \frac{(y_0 - C_{L0} + a)b}{2x} \tag{10}$$

$$C^* = y_0 + \frac{xq_{O_2}}{b} \tag{11}$$

The primary C_L versus time data obtained during a typical experiment with gas-phase composition changes during a batch culture of *A. terreus* in a 17-L fluidized-bed bioreactor are shown in Figure 2. The values obtained for the absorption step were as follows: $K_La = 26.64\ h^{-1}$ and $q_{O_2} = 0.4538$ mmol $g^{-1}\ h^{-1}$. The values obtained for the desorption step were $K_La = 24.84\ h^{-1}$ and $q_{O_2} = 0.4232$ mmol $g^{-1}\ h^{-1}$.

3. Materials and Methods

3.1. Microorganism and Culture Conditions. The microfungus used was obtained from the American Type Culture

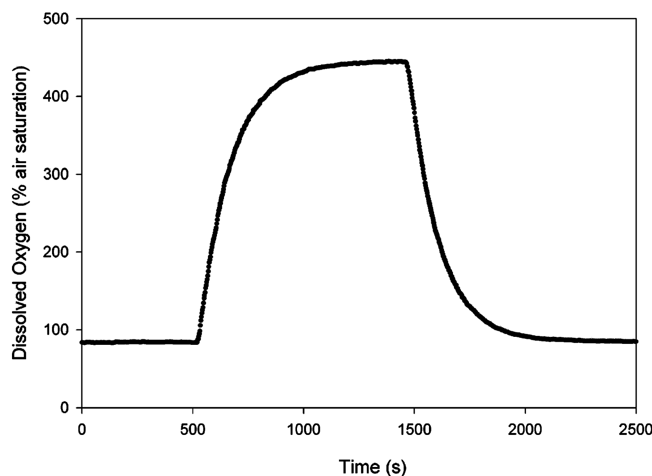

Figure 2. Primary data obtained using the proposed method in a batch culture of *A. terreus* in a 17-L fluidized-bed bioreactor.

Collection, as *Aspergillus terreus* ATCC 20542. Pelleted growth was promoted by manipulating inoculation conditions.[10] The culture medium was as detailed by Rodríguez Porcel et al.[10]

3.2. Stirred-Tank Bioreactor. The experiments were conducted in a 5-L working volume bioreactor (Bioflo III, New Brunswick Co., USA) with a vessel internal diameter, T, of 0.17 m, four equally spaced baffles, a rounded bottom, and a broth-height-to-vessel-diameter ratio of 1.4. Agitation was provided by two Rushton turbines with a D/T ratio of 0.38 and a W/D ratio of 0.18. Spacing between the impellers was $2D$, and the lower impeller was located at a distance D above the base of the tank. Agitation speed was 300 rpm. A pipe sparger aerated the culture at 1 vvm.

3.3. Fluidized-Bed Reactor. Fermentations were carried out in a fluidized-bed reactor with 20 L of total volume (17 L of working volume). The reactor vessel had a diameter of 0.155 m. The aspect ratio was 6. Gas was sparged at 1 vvm using a perforated plate with 150 holes of 1.5 mm diameter. This corresponded to a superficial aeration velocity of 0.015 m s^{-1}, or an approximate specific power input value of 150 W m^{-3}. The reactor was fitted with a jacket for temperature control. Fermentations were carried out at 28 °C. The top degassing zone of the fluidized-bed column had a jacket of its own, and this was held at 4 °C to prevent wall growth.

3.4. Biomass Concentration. The biomass (as dry weight) was determined by filtering a known volume of the broth through a 0.45-μm Millipore membrane filter, washing the cells with sterile distilled water, and freeze-drying the solids.

3.5. Dissolved Oxygen Measurements. Dissolved oxygen was measured using a polarographic Mettler Toledo electrode InPro 6100/220T. Electrode characteristics can influence the K_La measurements.[11] Therefore, the electrode dynamic response was characterized experimentally and found to be a first-order response with time delay. Thus, $C_L(t - t_d) = C_E(t) + 1/k\, dC_E(t)/dt$, where t_d is the delay time and k is the time constant. The dissolved oxygen readings were corrected to take into account the delay time ($t_d = 5.2 \pm 0.2$ at a 95% confidence level) and the time constant k ($= 0.064 \pm 0.004$ s^{-1} at a 95% confidence level).

The dissolved oxygen electrode was calibrated in a sterilized uninoculated broth. For this, the broth was first bubbled with nitrogen until a zero steady-state level of dissolved oxygen had been attained. This condition was used to set the zero reading. Subsequently, the broth was bubbled with pure oxygen to attain a steady-state saturation concentration of dissolved oxygen, and the reading was adjusted to the saturation value. The time constant k and the delay time t_d were characterized by instantaneously transferring the calibrated electrode from a steady state in the normal atmosphere to a well-agitated and aerated beaker of water that had attained a saturation level of dissolved oxygen. The resulting electrode response curve was measured with time from the instance of immersion until a steady-state reading was attained. The response curve modeled with the above-mentioned equation was fitted to the measured response curve using k and t_d as the fitting parameters. In view of the generally turbulent and steady hydrodynamic conditions during measurements, any effects of the liquid film at the surface of the electrode were disregarded, as is typical for this kind of work.[1]

For measurements using the classic dynamic method,[2] the gas flow was stopped and the decline in the dissolved oxygen concentration was monitored as a function of time. This desorption step lasted for 150 to 250 s. Aeration was always restored before the dissolved oxygen concentration had declined to <20% saturation, thus preventing possible damage to the biomass. The dissolved oxygen concentration was monitored during absorption until the concentration was close to saturation.

The measurements using the proposed method were carried out by changing the composition of the aeration stream from normal air (21% O_2 by vol) to pure O_2. The total mass flow rate of the aeration gas did not change. The dissolved oxygen concentration was monitored as a function of time, from a little prior to the instance of changed composition. Once the medium was near saturation with oxygen, the composition of the aeration gas reverted to that of air and the desorption step commenced.

4. Results and Discussion

The proposed method was first carefully compared with the conventional dynamic method in a stirred-tank bioreactor. The reproducibility of the measurements was evaluated in a biomass-free bubble column. Subsequently, the method was applied to a fluidized-bed reactor during culture of the filamentous microfungus *A. terreus*, which cannot be subjected to the conventional dynamic method because uninterrupted aeration is necessary for mixing the suspension and preventing settling.

4.1. Comparison with the Conventional Dynamic Method. The stirred-tank reactor is well-suited to measurements by the conventional dynamic method because mechanical agitation ensures mixing and prevents settling of the biomass during interruption of gas flow. Therefore, the proposed and conventional methods were compared in four separate experiments in a batch culture of *A. terreus*. The culture was close to the stationary phase during four consecutive days of measurement. Biomass concentration ranged from 6.6 to 7.2 g L^{-1}. The results obtained for the two methods are shown in Table 1.

As shown in Table 1, both K_La and q_{O_2} values determined by the two methods were essentially identical, proving that the proposed technique accurately measured these variables. The variability of the proposed method was actually lower than that of the conventional technique. The average value of K_La obtained with the proposed method was 20.3 ± 0.9 h^{-1}. In contrast, average K_La and standard deviation for data measured by the conventional technique were 19.6 ± 1.1 h^{-1}. These comparisons are for a 95% confidence level. Similarly, averages and standard deviations for the q_{O_2} measurements were 1.74 ± 0.03 mmol L^{-1} h^{-1} (proposed method) and 1.83 ± 0.04 mmol L^{-1} h^{-1} (conventional method).

4.2. Reproducibility Aspects. Further experiments were carried out in a bubble-column type of bioreactor to assess the

Table 1. Comparison of K_La Values and Specific Oxygen Uptake Rate (q_{O_2}) Measured by the Proposed Method and the Original Dynamic Method

	proposed method		proposed method (averaged)		classic dynamic method	
experiment	K_La (h^{-1})	q_{O_2} (mmol L^{-1} s^{-1})	K_La (h^{-1})	q_{O_2} (mmol L^{-1} s^{-1})	K_La (h^{-1})	q_{O_2} (mmol L^{-1} s^{-1})
1-a	20.3	1.99	19.7	1.94	20.16	1.82
1-d	19.1	1.88				0.00
2-a	20.0	1.97	19.6	1.93	18.10	1.82
2-d	19.2	1.90				0.00
3-a	20.2	1.98	19.7	1.93	19.01	1.86
3-d	19.1	1.88				
4-a	22.3	1.12				
4-d	22.4	1.18				

Table 2. Reproducibility of the K_La Measurements in Water in a Bubble Column

experiment	K_La (h^{-1})
1-a	48.7
1-d	55.7
2-a	51.1
2-d	54.2
3-a	52.7
3-d	51.2
average	52.3
confidence interval (95%)	±2.0

reproducibility of the proposed method. The bioreactor was filled with tap water, without any biomass, so that the consumption term could be disregarded. Use of this simpler system instead of a fermentation broth eliminated any potential variability because of possible changes in biomass concentration and properties. Three absorption−desorption cycles were used to obtain the K_La values shown in Table 2. The average K_La value of 52.3 h^{-1} had an error range of <4% at the 95% confidence level. Thus, the measurement error in the proposed technique is comparable to the typical error in conventional K_La measurements.[9]

4.3. Application to a Fluidized-Bed Bioreactor. With its excellent accuracy and reproducibility established, the proposed method was used to measure the K_La values at various times during a 10-day batch culture of *A. terreus* that produced the cholesterol-lowering drug lovastatin. Good oxygen transfer is critical to this fermentation, and the high biomass concentrations that are usually attained severely strain the mass transfer capacity of the system. The measured K_La, OUR, and biomass concentration data are shown in Figure 3. After inoculation with spores, the biomass concentration increased rapidly in the first 50 h to 3 g L^{-1}. Subsequently, the growth rate declined and the increase in biomass concentration was more gradual until the maximum concentration of 4 g L^{-1} was attained The gradual slowing of growth rate and attainment of a stationary phase were associated with depletion of nitrogen in the medium. The K_La was not affected by biomass growth (Figure 3), and this is explained by changes in fungal morphology and rheology, as discussed in detail previously.[12] The K_La value ranged between 24 and 29 h^{-1}.

The OUR declined as the growth rate slowed (Figure 3). OUR varied from an initial value of 1.9 mmol L^{-1} h^{-1}, at a biomass concentration of 1.3 g L^{-1}, to 0.72 mmol L^{-1} h^{-1} near the end of the fermentation when the biomass concentration was 3.3 g L^{-1}. This change was attributed to the onset of stationary phase in which active primary metabolism is slow.

5. Conclusions

The conventional dynamic method for the measurement of overall volumetric oxygen transfer coefficient was modified to eliminate the necessity of stopping the aeration as required in the conventional method for determining the oxygen consumption rate. The applicability of the dynamic method was, therefore, extended particularly to systems that use the aeration gas as the exclusive means of mixing. Furthermore,

• The proposed method was compared with the widely used conventional dynamic method, to prove that the two methods gave statistically identical values of K_La and OUR at a 95% confidence level.

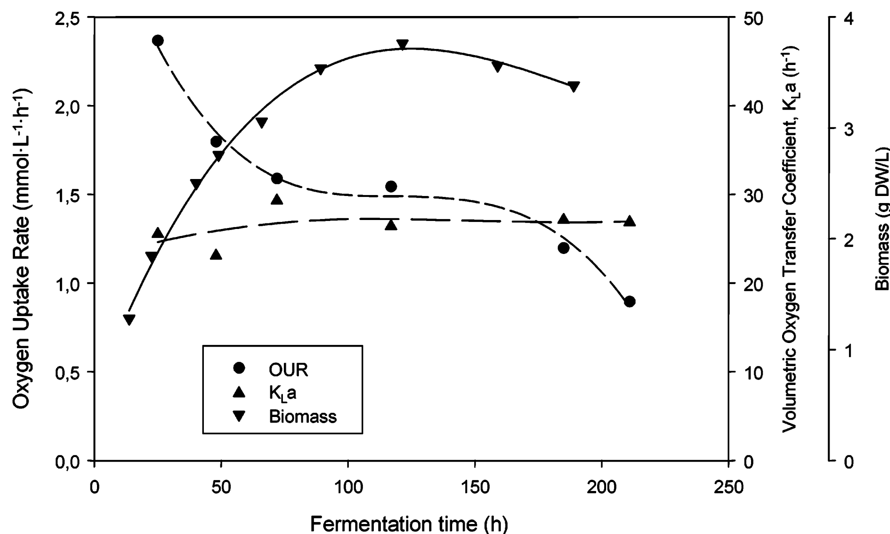

Figure 3. Volumetric oxygen transfer coefficient (K_La) and the biomass oxygen uptake rate (OUR) variation with time during a 10-day batch culture of *A. terreus* in a 17-L fluidized-bed bioreactor.

• The reproducibility of the measurements with the modified method was within ±4% of the average value at a 95% confidence level.

• The proposed method was demonstrated at the 17-L scale during batch culture of the microfungus *A. terreus* in a fluidized-bed bioreactor. The results obtained with the modified method during a 10-day period were consistent with the conventionally measured data.

The modified method presented is, therefore, a valuable tool for experimental measurements of K_La and oxygen uptake rates in bioreactors under a wide range of operating conditions.

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Nomenclature

C^* (mg L^{-1}) = equilibrium oxygen concentration in the liquid phase
C_L (mg L^{-1}) = oxygen concentration in the liquid phase
C_E (mg L^{-1}) = oxygen concentration measured by the electrode
D (m) = impeller diameter
K_La (h^{-1}) = volumetric oxygen transfer coefficient
k (s^{-1}) = electrode time constant
OUR (mmol L^{-1} h^{-1}) = oxygen uptake rate
q_{O_2} (mmol g^{-1} h^{-1}) = specific oxygen consumption rate (referred to g of biomass)
T (m) = tank diameter
t (s) = time
t_d (s) = electrode delay time
W (m) = impeller blade width
x (g L^{-1}) = biomass concentration

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