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

J. L. Casas López,‡* E. M. Rodríguez Porcel,† I. Oller Alberola,† M. M. Ballesteros Martin,‡
J. A. Sánchez Pérez,‡ J. M. Fernández Sevilla,‡ and Y. Chisti‡

Department of Chemical Engineering, University of Almería, 04120 Almería, Spain, and Institute of Technology and Engineering, Massey University, Private Bag 11 222, Palmerston North, New Zealand

A new approach is proposed for the simultaneous determination of the volumetric oxygen transfer coefficient ($K_{L,a}$) 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 $K_{L,a}$ 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 ($K_{L,a}$) and the oxygen uptake rate (OUR). Accurate knowledge of the values of $K_{L,a}$ 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 $K_{L,a}$ values in biological systems is difficult; hence, data measured in culture broths are necessary for various purposes.

Several methods are available for measuring $K_{L,a}$, but few of these are applicable to measurements in biological systems during operation. The well-known dynamic method is one of the most frequently used techniques for measuring $K_{L,a}$ 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 $K_{L,a}$. 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 $K_{L,a}$ 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 $K_{L,a}$, 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 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. A method involving variation of the composition of aeration gas has been used for determining $K_{L,a}$, but only in nonbiological systems that did not involve oxygen consumption.

The modified dynamic method presented in this work for simultaneous measurement of $K_{L,a}$ 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:

$$\frac{dC_L}{dt} = K_{L,a}(C^* - C_L) - xqO_2$$

(1)

In eq 1, $\frac{dC_L}{dt}$ is the accumulation of oxygen in the liquid phase, $K_{L,a}(C^* - C_L)$ represents the oxygen transfer rate from the gas to the liquid phase, $qO_2$ is the specific oxygen consumption rate, and $x$ is the biomass concentration, with the product $-xqO_2$ being the oxygen uptake rate (OUR).
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

$$C_L = -xqO_2 t$$

(2)

Thus, $-xqO_2$ (OUR) can be obtained as the slope of a plot of $C_L$ versus time. The specific oxygen consumption $qO_2$ 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:

$$C_L = -\frac{1}{K_La}\left(\frac{dC_L}{dt} + xqO_2\right) + C^*$$

(3)

Equation 3 is a rearranged form of eq 1. For a given biomass concentration and known $qO_2$, $K_La$ is obtained as the slope of a plot of $C_L$ versus $(dC_L/dt) + xqO_2$. 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 $(C_L^*-C_L)$. 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 $(C_L - C_L^*)$ 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 $qO_2$ 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 $xqO_2$, $C^*$, and $K_La$ remain constant, a new variable $\alpha$, defined by eq 4, is also a constant:

$$\alpha = K_LaC^* - xqO_2$$

(4)

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

$$\frac{dC_L}{dt} = \alpha - K_LaC_L$$

(5)

Equation 5 can be integrated as follows:

$$\int_{C_{L0}}^{C_{L1}} \frac{dC_L}{(\alpha - K_LaC_L)} = \int_0^t dt$$

leading to

$$\left(\frac{\alpha - K_LaC_L}{\alpha - K_LaC_{L0}}\right) = e^{-K_La\alpha t}$$

(6)

After substituting $\alpha$ (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:

**$C_L$**

\[\begin{align*}
C_L &= \left(C_0^* - \frac{xqO_2}{K_La}\right) + \left(C_{L0} - C_0^* + \frac{xqO_2}{K_La}\right) e^{-K_La\alpha t}
\end{align*}\]

(8)

\[\begin{align*}
C_L &= \left(C_1^* - \frac{xqO_2}{K_La}\right) + \left(C_{L1} - C_1^* + \frac{xqO_2}{K_La}\right) e^{-K_La\alpha t}
\end{align*}\]

(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 $y_0 = (C_0^* - xqO_2/K_La)$, $a = (C_{L0} - C_0^* + xqO_2/K_La)$ 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, $C^*$, $qO_2$, and $K_La$ can be readily calculated. Separate values are obtained for absorption and desorption steps. The equations used in calculating $qO_2$ and $C^*$ are as follows:

$$qO_2 = \frac{(y_0 - C_{L0} + a)b}{2x}$$

(10)

$$C^* = y_0 + \frac{xqO_2}{b}$$

(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 $qO_2 = 0.4538$ mmol g$^{-1}$ h$^{-1}$. The values obtained for the desorption step were $K_La = 24.84$ h$^{-1}$ and $qO_2 = 0.4232$ mmol g$^{-1}$ h$^{-1}$.

3. Materials and Methods

3.1. Microorganism and Culture Conditions. The micro-fungus used was obtained from the American Type Culture
the tank. Agitation speed was 300 rpm. A pipe sparger aerated
ducted in a 5-L working volume bioreactor (Bioflo III, New
corresponded to a superficial aeration velocity of 0.015 m s
m. The aspect ratio was 6. Gas was sparged at 1 vvm using a
working volume). The reactor vessel had a diameter of 0.155
in a fluidized-bed reactor with 20 L of total volume (17 L of
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. The
culture medium was as detailed by Rodriguez Porcel et al.

3.2. Stirred-Tank Bioreactor. The experiments were con-
ducted 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 Kₐ,a
measurements. Therefore, the electrode dynamic response was
characterized experimentally and found to be a first-order
response with time delay. Thus, \( C(t - \tau_d) = C(t) + 1/k \int_{t-\tau_d}^{t} dC_{E^-} / dt \) dt, where \( \tau_d \) is the delay time and \( k \) is the time constant. The
dissolved oxygen readings were corrected to take into account
the delay time (\( \tau_d = 5.2 \pm 0.2 \) 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
gas 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 \( \tau_d \) 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 \( \tau_d \) 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.

For measurements using the classic dynamic method, 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% O₂ by vol) to pure O₂. 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 conven-
tional 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 \( K_{l,a} \) and \( q_{O2} \) 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 \( K_{l,a} \)
obtained with the proposed method was 20.3 ± 0.9 h⁻¹. In
contrast, average \( K_{l,a} \) 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 \( q_{O2} \) 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
conducted in a bubble-column type of bioreactor to assess the
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.](image)
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.

**Acknowledgment**

This research was supported by the Ministerio de Ciencia y Tecnología (MYCT), Spain, FEDER project PPQ2000-0032-P4-02, and Plan Andaluz de Investigación PAI-III.

**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

$q_{O2}$ (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

**Literature Cited**


*Received for review July 1, 2005 Revised manuscript received November 7, 2005*