Biochemical Engineering Journal 199 (2023) 109042

Contents lists available at ScienceDirect

Biochemical Engineering Journal

journal homepage: www.elsevier.com/locate/bej

Minimally-disruptive method to estimate the volumetric oxygen mass transfer coefficient (k_La) for recombinant *Escherichia coli* fermentations

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ARTICLE INFO

Keywords:
Dynamic gas-out
Oxygen transfer rate (OTR)
Oxygen uptake rate (OUR)
Ambr250

ABSTRACT

In the biopharmaceutical industry, accurate prediction of the oxygen uptake rate (OUR) is critical to understanding cell health, where OUR is related to the oxygen transfer rate (OTR) and the culture dissolved oxygen (DO). Key to accurate OTR assessment is an accurate volumetric oxygen mass transfer coefficient (k_La) estimate, where k_La represents the oxygen driving force from gas to liquid phase. Common approaches to estimate k_La have significant limitations, such as disruptive to the culture by stopping the oxygen supply or the use of only cell-free buffered solution. Yet, it is well-known that cell secretions and additions (i.e., base and antifoam) can dramatically can affect k_La, and accumulate during the culture. This study describes a novel, minimally-disruptive method to estimate k_La by halving the gas flow rate periodically throughout the fermentation. This approach was used to estimate k_La at multiple times for two recombinant *Escherichia coli* strains cultured in ambr250 modular vessels. In one case, the duration of the halved gas flow was varied. In the second case, the effects of only cell secretions was examined. As the oxygen supply was only halved, the risk of culture loss was significantly lower compared to the dynamic gas-out method.

1. Introduction

Recombinant proteins are a significant part of the biopharmaceutical industry and diagnostics. These recombinant protein can be produced in bacteria, yeast, insect, plant, and mammalian cells [1–3]. As the demand for these therapeutic products and diagnostic enzymes grows, so does the need to increase product yields. Maintaining adequate dissolved oxygen (DO) levels is a criterial requirement to optimize bioreactor performance [4]. A key to DO maintenance is controlling the oxygen supply, given by the oxygen transfer rate (OTR). The OTR needs to exceed the needs of the cells, where the actual oxygen utilization by the cells is called the oxygen uptake rate (OUR) [4,5]. OTR is depend on the volumetric mass transfer coefficient, k_La, and the driving force for the exchange dictated by the Henry's Law and the culture DO [6]. Henry's Law predicts oxygen saturation solubility, or concentration, in aqueous solutions as a function of the Henry's constant, which is a function of temperature, and the oxygen partial pressure in the gas. Thus, it is common to enrich inlet air streams to fermenters with oxygen to control the DO, as this enrichment increases the oxygen saturation concentration and likewise then reduces the required k_La to maintain the OTR to support the culture's OUR [7–10]. The balance between OTR and OUR determines the culture DO, where DO can become too low if the OUR needs of the cells outpace the OTR capacity of the gassing and stir protocols [11]. However, k_La is sensitive to many culture parameters, such as antifoam additions, bioreactor shape, cell density, media characteristics, and stir speed [7,12]. Yet, it is common to obtain k_La using

Abbreviations: a, empirical constant dependent on the vessel configuration (unitless); b, empirical constant dependent on the vessel configuration(unitless); D_i, is the impeller diameter (m); DO^*, liquid oxygen saturation constant (86% saturation relative to sea level); DO, dissolved oxygen (% saturation); K, empirical constant dependent on the vessel configuration $\frac{kg}{m \cdot s}^{0.65} \frac{m}{s}^{0.43}$; k_La, volumetric oxygen mass transfer coefficient (h^{-1}); k_La_{CO2}, carbon dioxide volumetric mass transfer coefficient (h^{-1}); P, power (W); N, stir speed (rpm); N_P, the power number (unitless); OD, optical density, used as measure of cell density; OTR, oxygen transfer rate (g/L·h); OUR, oxygen uptake rate (g/L·h); q_{O2}, cell specific oxygen consumption rate (g O_2/g cell·h); ρ, the fluid density (kg/m^3); t, time (h, min, or s); T, temperature (°C or K); V, liquid volume (L or mL); v_S, superficial gas velocity (m/s); *X*, cell density (OD at 600 nm).

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https://doi.org/10.1016/j.bej.2023.109042
Received 4 May 2023; Received in revised form 11 July 2023; Accepted 18 July 2023
Available online 26 July 2023

buffered solutions instead of media, and without cells [5,11,13]. For these reasons, using a k_La estimated only in an aqueous buffer without cells can lead to inaccurate OTR and subsequently inaccurate OUR estimates, where OUR is often used to assess culture health [8,11,14,15].

Current OUR assessment methods for fermenters and bioreactors are divided into three approaches: 1) the dynamic gas-out method, 2) the global mass balance method, and 3) the stationary liquid mass balance method [9,13,15,16]. There are several other k_La methods in the literature for chemical reactors, which unfortunately, have limited application for systems with live cells [17,18]. Namely, these approaches employ chemicals, such as sodium sulfite (Na_2SO_3) or carboxymethyl-cellulose [19], that are not conducive to cell growth. Further, some of these methods require pressurization of the vessels that are above safety levels for disposable plastic vessels [19,20], such as the ambr250 bioreactor, or use gas flow rates that are too high for many standard and disposable vessels [21]. The dynamic gas-out method is the most commonly used approach to determine k_La prior to cell additions to the bioreactor often in a buffered solution. To estimate a culture's OUR using the dynamic gas-out method, the descending DO profile generated during the de-aeration phase is used. During the reaeration phase, k_La is obtained, which then can be used to calculate OTR [8,22]. In the dynamic gas-out method, the inlet gas (air/oxygen) flow rate is stopped and often replaced with nitrogen at the same flow rate [23]. The dynamic gas-out method is considered disruptive or invasive as it completely disrupts the oxygen supply to the cells [16]. The global mass balance method subtracts the inlet and outlet oxygen concentrations assessed by off-gas analyzers while maintaining a constant DO, thus estimates OTR, but not k_La [16,24,25]. The stationary liquid mass balance method uses the difference between the liquid oxygen saturation and measured DO to estimate OUR. The stationary mass balance method requires k_La to be known or estimated independently [16]. These standard approaches have several limitations, such as the invasiveness of oxygen stoppage, the need for two off-gas analyzers, or the reliance on an pre-determined accurate k_La value, respectively [5,11,12,16].

The standard OUR estimation methods all obtain or require k_La to estimate OTR and subsequently OUR. When direct k_La estimates are not available, it is common to extrapolate k_La using the van't Riet (1979) equation given by Eq. (1) and first proposed by Cooper et al. (1944) [26,27].

$$k_La = K\left(\frac{P}{V}\right)^a (v_S)^b \tag{1}$$

Where **P** is the power input (W), **V** is the liquid volume (m^3), $\boldsymbol{v_s}$ is the superficial gas velocity (m/s), and **K**, **a**, and **b** are empirical constants dependent on the vessel configuration [13,28]. Goldrick et al. (2018) used the van't Riet equation to calculate k_La to control glucose feeding used in tandem with off-line glucose measurements [26,28]. Due to latency and signal filtering, Goldrick et al. (2018) were forced to smooth the OTR values, thus losing finer details of the real-time OUR information [28]. Fontova et al. (2018) calculated OUR in real-time using a combination of the global mass balancing technique and stationary liquid mass balance; yet used a constant k_La value [11]. They noted this simplification caused errors in the OUR estimates late in the culture [11]. Doi et al. (2020) measured both the mass transfer rate for oxygen (k_La) and carbon dioxide ($k_La_{CO_2}$) using the dynamic gas-out method in a buffered solution without cells for several bioreactor configurations [13]. They determined k_La varied significantly across the tank sizes, agitation speeds and sparging rates [13]. Likewise, Aroniada et al. (2020) reviewed k_La estimation methods and concluded that existing mathematical models to estimate k_La have underlying errors due to the models not accounting for oxygen transport resistance in the oxygen-permeable membrane of the DO probe [7]. Thus, better k_La estimators are desired to improve the accuracy of OTR estimates and subsequent OUR estimates during a culture [7,10].

In this study, a minimally-disruptive k_La method is described for *Escherichia coli* fermentations in stirred tank vessels using a common minimal media. This method does not require complete disruption of the gas flow rate to estimate k_La from the reaeration curve and can be conducted multiple times during a fermentation. Additionally, this approach is amenable to disposable plastic vessels with limited overpressure capabilities. Specifically, the gas flow rate was halved at the beginning of the k_La measurement cycle, and k_La was determined from the reaeration dynamics. These studies were conducted in stirred tank fermenters, as this configuration is the most common in the biopharmaceutical industry. The effect of insoluble and oil phases on k_La were not considered in this study, as these factors are uncommon in biopharmaceutical production vessels. However, others have developed k_La estimate methods for air lift and pneumatics fermenters and systems with insoluble material and oil [21,29]. Data will be presented that investigated the duration required for the minimally-disruptive event to obtain statistical significant results. Only the a halved-gas flow rate approach was investigated, as it provided a sufficient number of data points during reaeration to calculate k_La. Results will be presented that highlight the recommended minimally-disruptive method duration and other considerations.

2. Materials and methods

2.1. Bacterial strains and plasmids

Two *E.coli* strains were used in these studies. *E. coli* MG1655 cells were obtained from the American Type Culture Collection (ATCC). *E. coli* MG1655 represents a K-strain. The plasmid pTVP1GFP, was a gift from A. Villaverde, and encodes the VP1 capsid of foot-and mouth disease [30] fused to green fluorescent protein (GFP) [31]. *E. coli* BL21 T7 Express lysY/I^q were obtained from IMCS (Irmo, SC), who obtained the cells from New England Biolabs (Andover, MA). *E. coli* BL21 T7 Express lysY/I^q represents a B-strain. The plasmid pJE1F was provided by IMCS has kanamycin resistance and expresses β-glucuronidase [32]. All strains were stored at − 80 °C as 1 mL working cell banks, frozen from exponential growing cells in the media to be used for subsequent experiments.

2.2. Culture conditions

Pre-cultures were grown in 250 mL shake flasks with 50 mL of modified Korz media [33,34] supplemented with 50 μg mL^{-1} ampicillin for *E. coli* MG1655 pTV1GFP and 25 μg mL^{-1} kanamycin for *E. coli* BL21 T7 Express lysY/I^q pJE1F. The cultures were grown overnight at 37 °C and 250 rpm. Cell densities (OD) were obtained using absorbance at 600 nm (Genesys 30 Spectrophotometer, ThermoFisher), where 1.0 OD results in 0.45 g dry cell weight per L.

2.3. Fermenter operation

The ambr250 modular system (Sartorius Stedim, Germany) was utilized for all experiments using Rushton impellers (part #001–2A13). Culture conditions were 37 °C, pH 7.0, an initial stir speed of 800 rpm, and a dissolved oxygen (DO) set point of 40% air saturation. The pH was controlled with ammonium hydroxide aqueous (28–30%). The DO was controlled by a proportional-integral-derivative (PID) control loop with one control level, stir speed varied from 400 to 3000 rpm (k_P = 10 unitless, t_I = 250 s, t_D = 0 s). Due to the size of the ambr250 vessels, stir speeds as high as 3000 rpm are necessary to match the power per volume (P/V) ratio of production vessels [35,36]. Additionally, over-pressurization must be avoided with these disposable plastic bioreactors, as the system will stop the gas flow if the pressure readings are ± 10 mbar atmospheric pressure. The vessels were inoculated to approximately 0.25 OD with 15 mL of exponentially growing cells into 135 mL Korz media [33,34] for a total volume of 150 mL. The gas flow was maintained at 100 mL min^{-1} which represents roughly a 0.66 vvm

(volume of gas per volume of liquid per minute). The gas flow rate of 100 mL min^{-1} is the maximum gas flow rate in the ambr250 modular system. During the k_La estimation minimally-disruptive events, the gas flow rate was halved to 50 mL min^{-1} (~0.33 vvm). The DO spot sensors within the ambr250 vessels were pre-calibrated at sea level (101.3 kPa) and 25 °C in water. These calibration conditions result in DO readings at local conditions (approximately 260 m) in the culture media without cells at 37 °C as 86% oxygen saturation. Thus, the oxygen saturation concentration [dissolved oxygen (DO*)] was taken to be 86%.

3. Theory and calculations

The minimally-disruptive k_La estimation method halves the gas flow rate periodically for short times during the fermentation and observes the DO response. Initially, when the gas flow is halved, the DO decreases. After the gas flow rate is returned to normal levels, the DO increases and returns to the DO levels anticipated, if the minimally-disruptive event had not occurred. The oxygen mass balance principles used for the minimally-disruptive k_La estimation method are similar to the dynamic gas-out method, in that the k_La can be determined from the rate of DO change during reaeration, since DO represents the balance between the supply and demand, OTR and OUR, respectively, as shown in Eq. (2).

$$\frac{d(DO)}{dt} = OTR - OUR \tag{2}$$

Where OUR is given by Eq. (3), where **X** is the cell density and q_{O2} is the cell specific oxygen uptake rate [22].

$$OUR = q_{O2} \cdot X \tag{3}$$

And OTR is given by Eq. (4), where **DO*** is the oxygen saturation concentration.

$$OTR = k_La(DO^* - DO) \tag{4}$$

At a steady state, the OUR and OTR are equal; however, when the system is perturbed by modulating the gas flow rates, it is possible to obtain OUR and k_La. Specifically, k_La is determined from the slope of the reaeration curve $(DO^* - DO)$ vs $\frac{d(DO)}{dt}$ as described by Eq. (5),

$$\frac{d(DO)}{dt} = k_La(DO^* - DO) - q_{O2} \cdot X \tag{5}$$

Where for the short duration of reaeration, OUR is assumed to be a constant due to the cell density (X) being relatively constant. For the standard dynamic gas-out method, the deaeration curve also provides OUR, since under gas stoppage OTR is zero. Further q_{O2} can be obtained from the rearrangement of Eq. (3), as the cell density can be obtained directly or estimated from off-line measurements [22].

The DO profiles for an example of the minimally-disruptive method and dynamic gas-out method are compared in Fig. 1. In this example set, the minimally-disruptive event was 2-min and the dynamic gas-out method stopped the air/oxygen gas flow for 20 s and switched to a 100% nitrogen gas sparge, adapted from profiles shown in Tribe et al., 1994 [37]. Despite the longer gas flow disruption, the minimally-disruptive event did not cause a significant DO drop (Fig. 1**A**). Also, this approach resulted in a slow non-linear decrease, because OTR was not suddenly zero. For the dynamic gas-out method, a very sharp DO is observed with a linear profile, and the system commonly reaches 0% DO (Fig. 1**B**).

4. Results and discussion

4.1. k_La estimate sensitivity to duration for the minimally-disruptive events throughout a batch culture

To determine conditions that best allowed for k_La estimates, the time interval (duration) for the minimally-disruptive event was investigated. These minimally-disruptive event durations needed to be suitably long to provide a sufficient number of data points to be collected during the reaeration period to allow for a statistically meaningful slope calculation. For the ambr250 modular system, DO and aeration rate data were automatically collected at 6 s intervals. The minimally-disruptive event duration also needed to be sufficiently short to maintain the DO above a critical DO level. The critical DO level for *E. coli* has been approximated as 0.35 mg L^{-1} [38] or 0.02 atm [39], which correspond to approximately 5% saturation in water or 2% saturation in media at 37 °C, respectively. The DO in this study was maintained above 14% saturation, thus well above the reported critical DO values for *E. coli*.

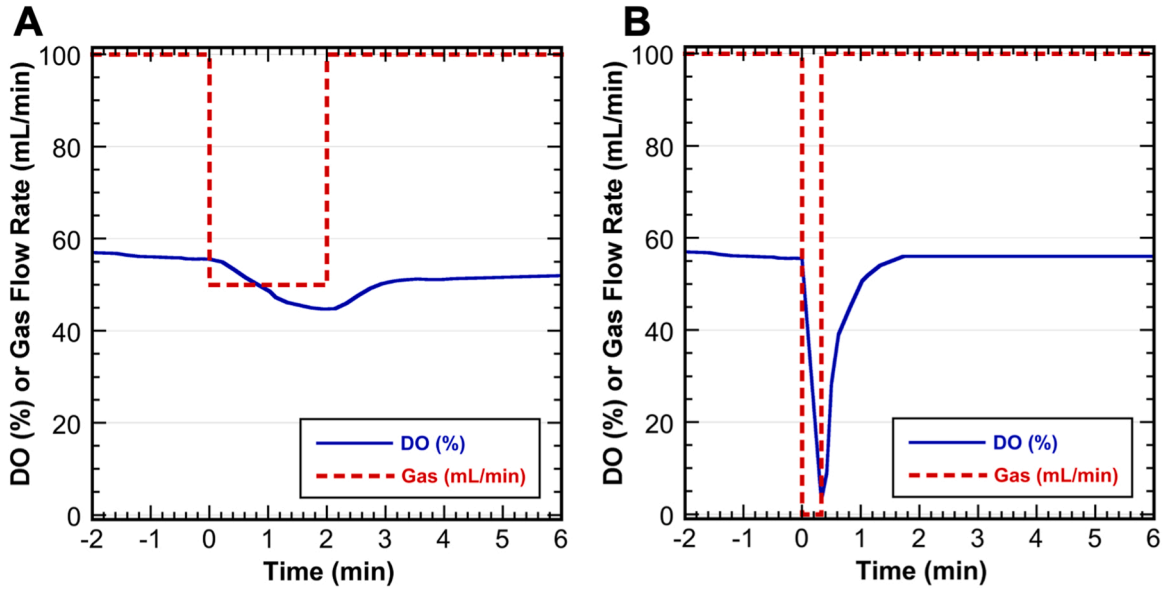

Fig. 1. Comparison of DO and gas rate flow profiles for the minimally-disruptive method and the dynamic gas-out method. A) Minimally-disruptive method, B) Dynamic or disruptive method. The DO (% saturation) profiles are shown as solid lines. The gas flow rate (mL/min) profiles are shown as dashed lines. DO and gas flow rates profiles in B) adapted from Tribe et al. [37].

E. coli MG1655 pTV1GFP (a K-12 strain) were used to investigate the effects of the minimally-disruptive method to obtain k_La. The minimally-disruptive event durations examined were 2-, 3-, 4-, and 5-minute and taken periodical throughout the fermentation. Fig. 2 shows the cell density (OD) profiles for the variable duration culture on both linear and logarithm scales. As can be observed on the logarithm curve (Fig. 2 inset), there was a slight lag phase for 1 h, followed by a three hour exponential phase and 2-h stationary phase. Despite the repeated lower aeration rate during the minimally-disruptive events, these repetitive gas flow decreases did not hinder exponential growth, as the exponential growth rate was constant for three hours. The final OD obtained was approximately 2.0 OD, which is expected for a batch culture in minimal media for this K-strain. Further, this growth profile is consistent with previous published work for this stain/plasmid/media combination when adjusted for different inocula ODs and initial glucose concentrations [10,34,40]. Additionally, the DO was never below the critical DO for *E. coli* [38].

Prior to each minimally-disruptive event, the stir speed was increased by 100 rpm approximately 20 min prior to an event and the DO PID control loop was suspended. The stir speed adjustment ensured the DO would not go below 14% saturation. In earlier culture experiments it was observed that 30-s and 1-min event durations were not sufficiently long to obtain a significant DO change, nor resulted in enough time points during reaeration to confidently calculate k_La (Data not shown). In the current work, only the 2- to 5-min events were compared.

For visualization, a normalization procedure was used for all seven reaeration profiles, such that the curves can be displayed together. These reaeration curves were normalized to DO value just prior to the minimally-disruptive event and an estimated OD at the event. Cell densities (ODs) for an event were estimated using an exponential fit for the growth rate determined using the offline measurements immediate prior and after the event. This normalization results in a ΔDO/cell density (%/OD) vs time curve (Fig. 3**A**), which shows both the deaeration and reaeration curves. The deaeration curve are not linear, as would be observed for the dynamic gas-out method. For both the minimally-disruptive and the dynamic gas-out methods, the non-linear reaeration curves are used to estimate k_La. It should be noted the DO level after returning to steady-state is lower than the initial DO level. This is a result of the suspended DO PID control and the constant stir speed. The observed steady-state DO levels correspond to the anticipated DO level had the event not occurred. Fig. 4 highlights this phenomena for two events (note the orange line shows the predicted DO profile without an event). The k_La value is determined from the slope of the $(DO^* - DO)$ vs $\frac{d(DO)}{dt}$ curves (Fig. 3**B**) and these values are reported in Table 1 along with k_La using the van't Riet equation and appropriate vessel constants.

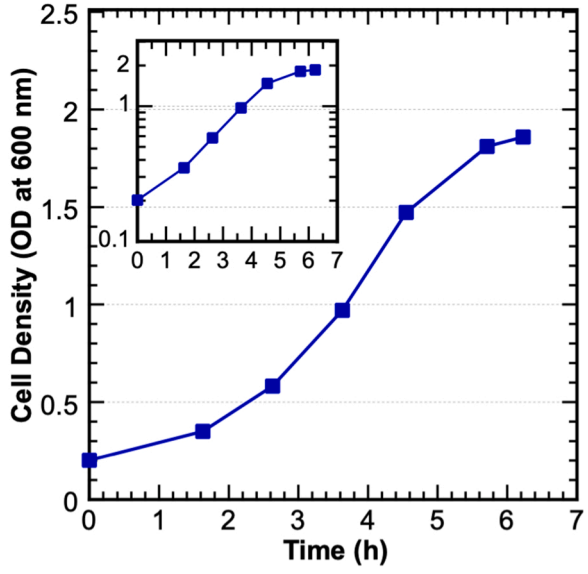

Fig. 2. Growth profiles for MG1655 pTV1GFP using the minimally-disruptive method with variable event durations.

To obtain the ambr250 vessel constants for the van't Riet equation, k_La values provided by Sartorius in the ambr250 manual for stir speeds between 1900 and 4200 rpm and aeration rates between 1.0 and 1.5 vvm, eight k_La data sets in all, were used. The vessel constants **a, b**, and **K** were obtained from linearization of the van't Riet equation, as shown in Eq. (6) as

$$\ln(k_La) = \ln(K) + a \bullet \ln\left(\left[\frac{N_P\rho D_i^5}{V}\right]N^3\right) + b \bullet \ln(v_S) \quad (6)$$

The $\left[\frac{N_P\rho D_i^5}{V}\right]$ term was considered a constant for the ambr250 vessels, since $N_P\rho D_i^5N^3$ is the power (P), and N_P is the power number (unitless), ρ is the fluid density (kg/m^3), and D_i is the impeller diameter (m). It was assumed that these stir speeds would resulted in a Reynold's number over 2000, such that N_P is a constant [41]. The van't Riet method only requires aeration rates and stir speeds for a particular vessel geometry to obtain these constants [26]. The constants for the ambr250 modular vessel with Rushton impeller were determined to be **a** = 0.65 (unitless), **b** = 0.43 (unitless), and $\mathbf{K} = 0.000125\left(\frac{kg}{m\bullet s^3}\right)^{0.65}\left(\frac{m}{s}\right)^{0.43}$ or $\left(\frac{kg^{0.65}}{m^{0.22}\bullet s^{2.38}}\right)$.

As expected, the k_La generally increased with stir speed for the minimally-disruptive method; however, later in the culture the minimally-disruptive k_La estimate and van't Riet equation methods diverge. The cause of this divergence was the result of two additions to the cultures to control the pH and foam. Namely, a significant base addition occurred at about 4.5 h (0.14 mL 28% ammonium hydroxide) and at 5.2-h antifoam was added. For both cases, the k_La obtained using the minimally-disruptive method was lower than predicted by the van't Riet question, which has been observed experimental by others [8]. In this study, the k_La estimate was unchanged between the two 5-min tests due to no significant additions, yet the OUR was much lower due to glucose depletion for the second 5-min event. These results indicate that the minimally-disruptive method is capable of distinguishing OTR and OUR, since k_La estimates do not solely depend on the stir speed and aeration rate (v_S). It was also determined that 2-min deaeration events was sufficient to obtain enough data points when sampling at 6-s intervals.

4.2. k_La estimates using only 2-min minimally-disruptive events throughout a batch culture

To validate the minimally-disruptive k_La estimator using only 2-min durations, *E. coli* BL21 pJE1F were cultured in the ambr250 modular. The growth profile for *E. coli* BL21 pJE1F is shown in Fig. 5. To better highlight the lag and exponential phases, the cell density vs time is shown on both linear and logarithm scales. As can be observed on the logarithm curve (Fig. 5 inset), there was a significant lag phase of about 2 h, followed by a 2-h exponential phase and 1-h stationary phase. The final OD obtained was approximately 2.0 OD, which is expected for a batch culture in minimal media for this strain with only 2 g/L glucose in the media. Further, this growth profile is consistent with previous published work for this stain/plasmid/media combination when adjusted for different inocula ODs and initial glucose concentrations [42]. Again, the DO never went below the critical DO for *E. coli* [38,39]. As this B-strain is known to have a higher than normal cell lysis potential [42], a large antifoam addition (~1 mL) was added to the media prior to the culture inoculation. This might have contributed to the longer lag phase.

The k_La estimates for the *E. coli* BL21 pJE1F fermentations, using the minimally-disruptive method, where no large base or antifoam additions were required, resulted in similar trend to the van't Riet equation

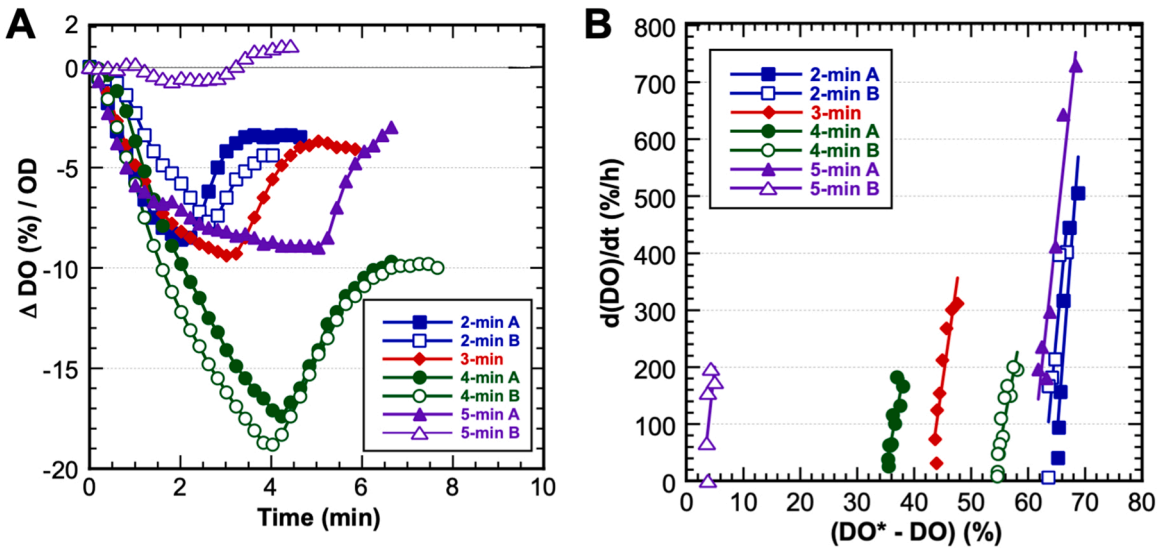

Fig. 3. The k_La estimates using the minimally-disruptive method for *E. coli* pTV1GFP in the ambr250 modular bioreactor. A) Reaeration curves, where the change in DO was normalized to the OD at the beginning of the test; B) k_La estimates were obtained from the slope of the difference between DO and DO* versus the time derivative of the DO change. Replicate event durations are indicated by A and B.

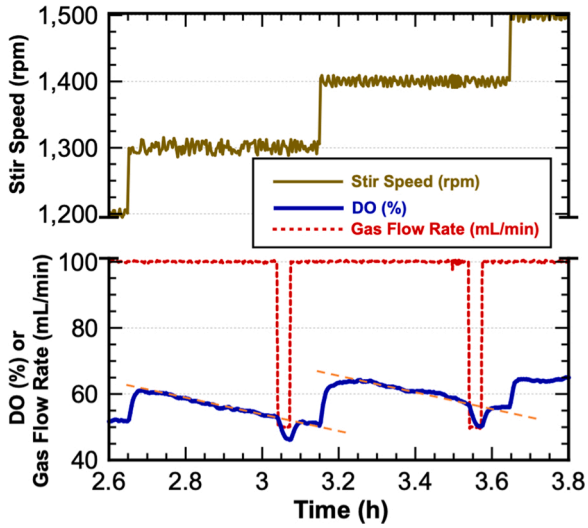

Fig. 4. Effect of a constant stir speed on DO profiles prior to and after two minimally-disruptive events. The stir speed profile is shown in the upper panel. The gas flow rate changes are shown for two events, while the DO responses are also shown in the lower panel. The predicted DO profile, had the minimally-disruptive event had not occurred, is highlighted by the dashed orange line.

method (Table 2). Interestingly, the k_La estimates were higher using the minimally-disruptive method in media and with cells compared to the dynamic gas-out methods obtained for phosphate buffered saline (PBS) extrapolating the van't Riet equation constants to the current conditions. Based on Garcia-Ochoa and Gomez [8], k_La increases significantly with ion concentration, which would occur due to media additions and cells secretions due to cell lysis [8]. Additionally, this B-stain is prone to cell lysis [42], much more than the K-stains, such that the k_La estimates would be expected to be higher relative to the van't Riet equation method, and this difference would increase with time, which agrees with the observation for the minimally-disruptive method.

The minimally-disruptive method k_La estimates were compared to the dynamic gas-out method values obtained with PBS in the ambr250 bioreactors and provided by the manufacturer. Fig. 6 summarizes the k_La estimates for the *E. coli* MG1655 pTV1GFP and *E. coli* BL21 pJE1F

Table 1
Estimated k_La values using the non-disruptive half-gas flow rate method with variable event durations compared to k_La estimated using the van't Riet equation and calculate vessel parameters **a, b**, and **K** and gas flow at 0.33 vvm.

Culture Time (h)	Half-gas flow rate event duration	Calculated OD	Stir speed (rpm)	k_La estimated with van't Riet (h^{-1})	k_La estimated with study method (h^{-1})
2.29	4-min A	0.503	800	36	52
2.79	4-min B	0.635	800	36	53
3.79	3-min	1.04	1200	78	89
4.29	2-min A	1.26	1200	78	132
4.78	2-min B	1.51	1400	106	107[a]
5.29	5-min A	1.65	1600	137	93[b]
5.79	5-min B	1.70	1600	137	92

[a] Base addition prior to this event
[b] Antifoam addition prior to this event

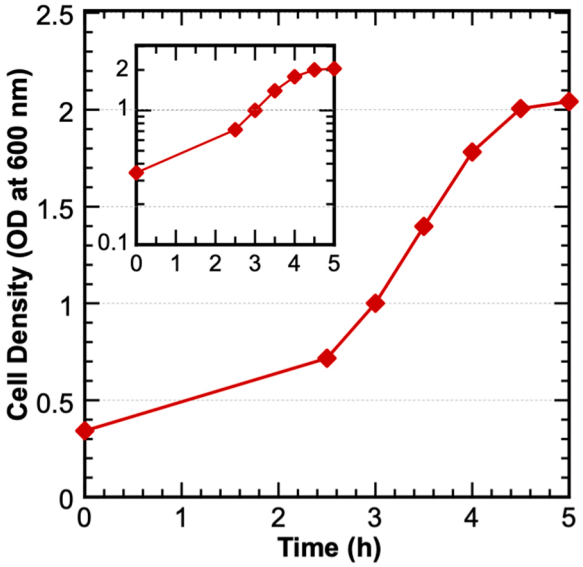

Fig. 5. Growth profiles for *E. coli* BL21 pJE1F using the minimally-disruptive method with 2-minute event durations.

Table 2
Estimated k_La values using the non-disruptive half-gas flow rate method with 2-min durations compared to k_La estimated using the van't Riet equation and calculate vessel parameters **a, b**, and **K** and gas flow at 0.33 vvm.

Culture Time (h)	Half-gas flow rate event duration	Calculated OD	Stir speed (rpm)	k_La estimated with van't Riet (h^{-1})	k_La estimates with this study method (h^{-1})
2.5	2-min	0.717	1300	92	91
3.0	2-min	1.0	1400	106	114
3.5	2-min	1.40	1500	121	141
4.0	2-min	1.78	1600	137	164
4.5	2-min	2.01	1700	155	196

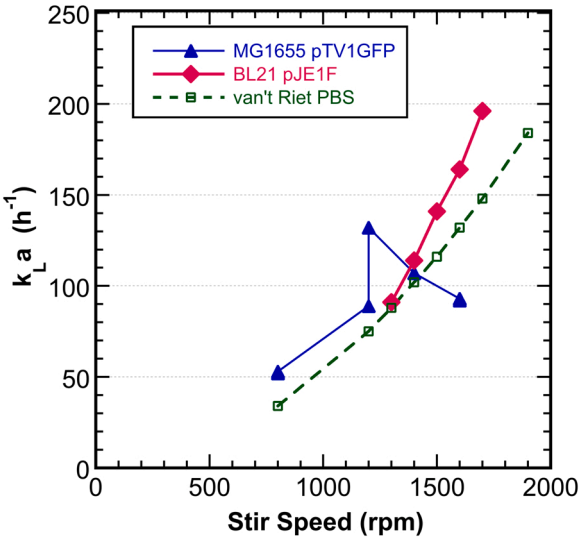

Fig. 6. Minimally-disruptive k_La estimator method used for two *E. coli* strains in ambr250 modular bioreactors. The k_La estimates versus stir speed for *E. coli* MG1655 pTV1GFP (variable duration, blue triangles ▲), *E. coli* BL21 pJE1F (2-min duration, red diamonds ◆), and extrapolation of the van't Riet equation fit of ambr250 bioreactor data obtained for 1.0 and 1.5 vvm ranging from 1900 to 4200 rpm stir speeds; data provided by manufacturer (green, unfilled squares ■).

fermentations, using the minimally-disruptive method. Also shown are the k_La values obtained from extrapolation of the van't Riet equation to the gas flow of 0.33 vvm and the experimental stir speeds (800–1700 rpm). Since the *E. coli* MG1655 pTV1GFP culture included significant base and antifoam additions, the non-linear correlation between k_La is readily observed. For the *E. coli* BL21 pJE1F cultures, a fairly linear correlation was observed for k_La with stir speed; however, the measured k_La slope is higher than that predicted by the van't Riet equation. This difference in k_La values is expected, since cell secretions would increase k_La, which is not accounted for in the van't Riet equation. The capability to re-evaluate k_La in real-time allows for better estimates of OTR, which will allow for more accurate OUR estimates, where OUR is an indicator of cell metabolism. Future work will focus on the application of the minimally-disruptive method to high-cell density *E. coli* cultures and mammalian cell cultures in the ambr250 modular and ambr250 HT bioreactor system.

5. Conclusions

In this study, a novel, minimally-disruptive k_La estimator, is described. The minimally-disruptive method is minimally-disruptive since the oxygen/air supply gas flow rate is never stopped, only reduced. And, the stir speed is increased prior to an event to compensate, such that the DO stays well above the critical DO for *E. coli*. Since the DO remains above the critical value, it is possible to periodically query the system and obtain near real-time k_La estimates. It was determined that a 2-min event duration was sufficiently long to deplete the DO and provide enough data points during the reaeration to confidently calculate k_La for *E. coli* cultures. This duration assumes a sampling rate on par with the ambr250 system at 6 s intervals in order to obtain as sufficient number of data points for the linearization. Further, the effects of base and antifoam additions on k_La were measurable using this minimally-disruptive method. This minimally-disruptive method was able to determine k_La for two *E. coli* strains with slightly different growth characteristics in a minimal media. Future work will focus on the application of this minimally-disruptive method to mammalian cell cultures and high-cell density *E. coli* cultures.

Funding

This work was supported by the US National Science Foundation [OIA-1736123] IIP-1624641, and EEC-2100442.

CRediT authorship contribution statement

Elliot Mercado: Conceptualization, Methodology, Investigation. **Sarah Mbiki**: Data Analysis, Writing – original draft. **Sarah W. Harcum**: Data Analysis, Writing – review & editing, Supervision. **Jordon A. S. Gilmore:** Writing – review & editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data can be made available upon request to the corresponding author.

Acknowledgments

We thank E. García-Fruitós and A. Villaverde at the Universidad Autónoma de Barcelona for donating the plasmid pTVP1GFP and Integrated Micro-Chromatography Systems, Inc (IMCS) in Irmo, SC for providing the E. coli BL21 pJE1F strain. We acknowledge Tom Caldwell for providing technical assistance with the E. coli cultures.

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