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An Accurate Measurement Technique for the Biological Oxygen Uptake Rate

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Received: 2 January 2025; **Revised:** 31 October 2025; **Accepted:** 5 November 2025

Abstract: For any wastewater treatment aeration tank, the paper proposes an accurate technique to deal with the Oxygen Uptake Rate (OUR) measurements. Since it measures the rate at which oxygen is used (in mg O_2/L/hour), it is a useful tool to evaluate process performance, aeration equipment, and the biodegradability of the waste. Unfortunately, the literature abounds with examples of inconsistent measurement results. The manuscript observes that if a sample of mixed liquor is withdrawn from an aeration tank operating at low Dissolved Oxygen (DO) (dissolved oxygen), the OUR measured in the sample after shaking (or other means of perturbation) will be higher than the true OUR which is limited by oxygen supply. The composition of a sample of activated sludge being analyzed is continually changing, making it necessary to obtain measurements as quickly as possible at the site of the aeration basin. To alleviate the many problems in measurement, the proposed method using water dilution with saturated DO may give a more accurate measurement than the current standard method as described by the American Public Health Association (APHA). The discrepancy in the new method between the measured and the calculated $SOTR_{pw}$ is in the assumed mole fraction of the exit gas of 0.19 which is reasonable but still based on guesswork. However, the discrepancies in the conventional method are that, the measured value of R is incorrect because of the inherent shortfall in the APHA (Biological Oxygen Demand (BOD) bottle shaking) technique, and it is more realistically given by the modified Eq. (2-3), which is originally stated as for a batch process provided by the American Society of Civil Engineers (ASCE) Guidelines, ASCE/Environmental and Water Resources Institute (EWRI) 18-18 recently published; and secondly, the incorrect driving force at the steady state, making the OTR at test conditions erroneously high. With a more accurate measurement of the OUR, it may be justified to modify the fundamental equation for oxygen transfer in a respiring system, as applied to the example given by the Guidelines. The specific content of the revised formula is proposed to be for a batch process.

***Keywords*:** Oxygen Uptake Rate (OUR), microbial respiration rate, oxygen transfer, gas depletion, measurement technique

1. Introduction

Aerobic metabolism rates are frequently reflected in oxygen consumption rates, although inorganic carbon remineralization and abiotic reoxidation of reduced species can also contribute to oxygen consumption rates. This paper focuses on the former type of oxygen consumption; hence the technique is primarily related to the biological (bacterial) uptake rate only. Oxygen Uptake Rate (OUR) is the rate of microorganism oxygen consumption per unit time and is one

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DOI: https://doi.org/10.37256/est.7120266338

of the few accessible parameters for quantifying the metabolism rate of the activated sludge in a wastewater treatment plant. Therefore, it is vital that it be measured correctly [1]. However, it is possible that the point at which the Dissolved Oxygen (DO) becomes limiting may itself be a function of the OUR. In other words, if a graph of OUR vs DO is made as shown in Figure 1, one may find different points at which the OUR starts to drop off as the DO goes from close to saturation to lower levels. One problem with the Biological Oxygen Demand (BOD) bottle type of OUR measurement [2] (method 2710B) is that it can only be used in a system that is not DO limited (typically assumed to be about 2 mg/L or more). If the system is running at low DO and is DO limited, as soon as the sample is aerated and given sufficient oxygen, the OUR will increase since the restriction has been removed. The measured OUR will therefore be erroneously high. It would be insightful to try the proposed dilution vs. the "shake it up" aeration approach to avoid shearing the floc, which may increase the OUR artificially, thus jeopardizing the true measurement in the aeration tank. In any bioreactor, when the level of dissolved oxygen in the medium falls below a certain point, the specific rate of oxygen uptake becomes dependent on the oxygen concentration in the liquid. Oxygen uptake rate and DO are then inversely proportional to each other. The problem with low DO concentration is well explained in [3] as well as in [4].

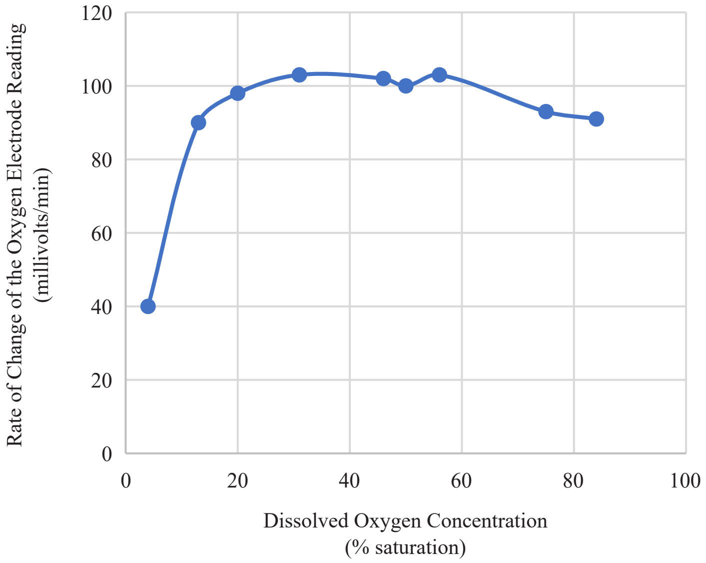

Figure 1. Effect of dissolved oxygen concentration on the rate of oxygen utilization by resting cells of *Pseudomonas ovalis* with no aeration at pH 7.0 and 25 °C [6]

The oxygen uptake rate is not solely dependent on dissolved oxygen levels, but the rate of oxygen consumption is affected by the DO levels. The Dissolved Oxygen Uptake Rate (DOUR) test measures the respiration rate of organisms. Since it measures the rate at which oxygen is used (in mg O_2/L/hour), it is a useful tool to evaluate process performance, aeration equipment, and the biodegradability of the waste. The oxygen uptake rate is one of the fundamental physiological characteristics of culture growth and has been used to optimize the fermentation process.

It is generally believed that the DO level only affects the uptake rate *R* (rate of oxygen consumption) when it reaches a critical level, above which *R* is unaffected by the DO concentration. About the inverse proportionality between *R* and DO in Waste Water Treatment Plants (WWTPs), it may be that by stopping the waste flow to the aeration basin being tested, and continuing the sludge recirculation system, the activated sludge will quickly reach endogenous metabolism and will have a uniform oxygen demand rate over the aeration system. The uniformity of oxygen uptake rate is a function of mixing characteristics and uniformity of oxygen supply, not the rate itself (be it high or low). In the well mixed basin of a Continually Stirred Tank Reactor (CSTR), the Mixed Liquor Suspended Solids (MLSS), substrate, and DO are all equal at any point in the tank regardless of the rate of oxygen uptake.

Since the oxygen uptake rate *R* will be relatively low, the DO in the aeration basin will be high. The DO also depends on the aeration rate relative to the oxygen uptake rate. Therefore, even at a low oxygen uptake rate, the DO can be low if the aeration rate is low. Conversely, a high OUR can exist with a high DO if the aeration rate is high enough [5]. In a certain sense, the two factors (OUR vs. DO) are inversely proportional to one another. In American Public Health Association (APHA) [2], the recommended method is the BOD bottle method, which is a standard approach to determine the R value of a sample of activated sludge. According to the American Society of Civil Engineers (ASCE), following the same terminologies, at steady state, the C_R value in the proposed new equations will be lower than (and possibly much lower than) the C_R value obtained using the ASCE relationships. Assuming one can develop a reliable method for determining the OUR that is reasonably accurate, one should be able to determine which oxygen transfer relationship is correct (or more precisely, which best fits the data).

To alleviate the many problems associated with measurement, the proposed method in this manuscript, using water dilution with saturated DO, may provide a more accurate measurement than the current standard method using a sample shaking technique as described in APHA [2]. This APHA method has a fatal drawback in that it can severely overestimate the consumption rate when the sample must be aerated to give a linear relationship in a plot of DO vs. % saturation. According to Figure 1 [6], when the DO concentration is above 17% (say, 1.4 mg/L for a saturation concentration C^* of 8.18 mg/L), the rate of oxygen utilization for the resting cells of Pseudomonas ovalis, in terms of the change in the oxygen electrode reading, becomes 100 (i.e., the rate of change of R is zero). R becomes constant until the sample reaches full saturation.

Conversely, when the sample begins with a high DO concentration, say 5 mg/L, R remains constant until the DO reaches the critical value of 1.4 mg/L, beyond which the rate of decline will no longer be linear. This scenario is true provided that the initial DO is not artificially aerated by shaking or injecting DO molecules as in a respirometer. Unfortunately, this is the recommendation of the Standard Methods [2], which advises increasing the initial DO level of the sample prior to measuring the DO rate of decline when the sample only has a minimal DO level because the DO concentration is low in the aeration basin from which the sample is collected.

Based on this finding by Bennett and Kempe, the value at which the rate of oxygen utilization becomes dependent on DO is defined as the critical DO concentration, which is around 2 mg/L in the mixed liquor. When the DO is above such a concentration, the utilization rate is then not dependent on the sample DO concentration. However, Kalinske [7] indicated that many factors affect the oxygen utilization rate, R, including high concentrations of living organisms and high turbulence levels, as well as the level of organic substrates present in the sample. High turbulence should have the same effect as shaking or other types of perturbation in a sample bottle, leading not only to a higher DO but also to a higher R value. The question is whether the higher R value is a consequence of the higher DO, given a constant organic substrate level and a fixed number of microbes, or whether it is due to other factors, such as agitation and excitation of the organisms from shaking the sample, which causes a higher respiration rate during the attempt to increase the DO, as stipulated in APHA [2].

2. Material and methods—proposed dilution method

If it were the latter, the remedy could be to simply mix two BOD bottles of equal volumes, one containing the sample with the microbes and food, and the other containing only pure water but already pre-aerated to a high DO concentration, and then measuring the resulting slope of the linear decay curve. The decay curve should show good linearity, which would represent the oxygen uptake rate as a negative slope. The respiration rate of the microorganisms in the mixture, which when multiplied by a factor of two should then give the *in-situ* R value in the aeration basin.

By this composite sampling method there should be no need to worry that "as soon as the sample is aerated and given sufficient oxygen, the OUR will increase since the restriction (limited DO) has been removed" because the original sample itself is not aerated by agitation as APHA [2] stipulates, but only by mixing two fluids together to obtain a uniform DO concentration.

In the article by Doyle et al. [8], an interesting method of testing for alpha was suggested. It appears that it may be possible to use the dilution method suggested in the article to test the determination. By first aerating a tank of pure water to an elevated DO (say, 7 mg/L) and then, upon stopping the aeration, gradually and carefully pouring a sample of known volume of the activated sludge mixed liquor into the tank, and gently mixing them together, it may be possible to measure the slope of the DO decline curve under quiescent conditions, thereby eliminating the second possible explanation for the cause of the increased OUR measurement. If the sample has been diluted to 50% by the tank, the resultant slope should then be multiplied by 2 to get the true OUR. This result should then be compared with the steady-state column test with an *in-situ* measurement as recommended by the ASCE/EWRI 18-18 Guidelines [9].

Alternatively, the mixed liquor can be continuously pumped into the test tank from a position within the existing aeration basin using a displacement pump until a set known volume is withdrawn. This should give the same oxygen depletion curve as the steady-state test, allowing the slope of the curve to be used as a measurement of the microbial oxygen uptake rate. To avoid any substrate-limiting effect, the test should be performed *in situ* as quickly as possible, just like the off-gas column test.

2.1 *Proposed steps for the dilution method*

i. Aerate a tank of pure water of 4 to 6 L volume to an elevated DO, say to 7 mg/L. A typical bench-scale aeration unit is shown in Figure 2 ([10], Figure 7.5).

Figure 2. A typical bench-scale aeration unit (image from Armfield Ltd.)

ii. Collect a sample of mixed liquor to be evaluated in a container of approximately 4 to 6 L, as shown in Figure 3 ([10], Figure 7.6b). The vessel can be made of a simple plastic bucket, flexible enough to contain a sufficient volume of wastewater directly withdrawn from the aeration tank at any suitable extraction point, which generally should correspond to the locations of the DO probes used for the aeration tank.

iii. Upon stopping the aeration, gradually and carefully pour a sample of a known volume of the activated sludge mixed liquor into the tank, as shown in Figure 4 ([10], Figure 7.6c). The bespoke aeration unit, as shown in Figure 2, should be equipped with a calibrated fast-response DO probe and a stirring mechanism.

iv. Then gently mix them together by the mechanical stirrer until mutual dilution is complete as shown in Figure 4 ([10], Figure 7.6 c).

v. Immediately measure the DO by the Winkler Method or by using a calibrated fast-response DO probe with a probe time constant of less than $0.02/K_La$. Start the timing device after the meter reading has stabilized. Record the corresponding DO data at time intervals of less than one minute, depending on the rate of consumption. Record data over a 15-minute period or until the DO becomes limiting, whichever occurs first.

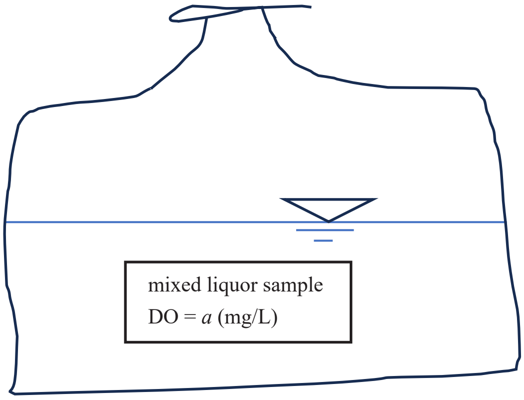

Figure 3. Sample of mixed liquor of a known volume in a container

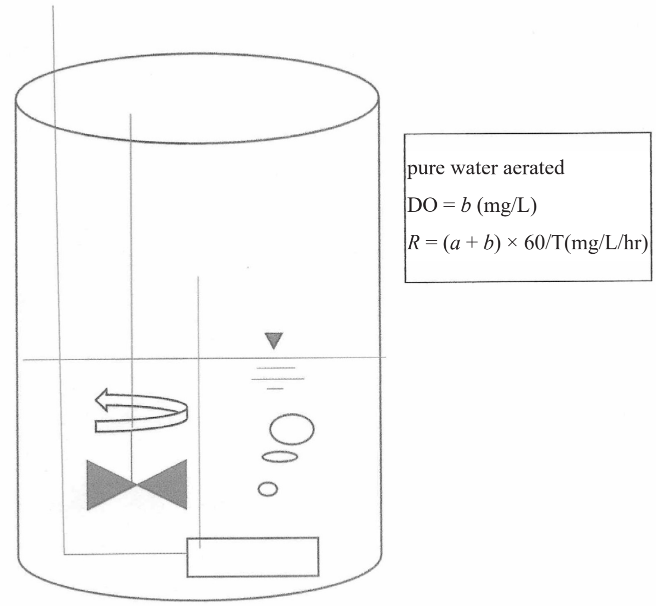

Figure 4. Aeration tank of same volume of pure water

vi. Plot the DO versus time (T) on a graph similar to that shown in Figure 5 ([10], Figure 7.6d), and calculate the slope by applying a linear least-squares regression to fit a straight line through the data points. The oxygen probe may not be accurate when the DO is below 1 mg/L. A low DO concentration (< 2 mg/L at the start of the test) may limit oxygen uptake by the biological suspension and is indicated by a decreasing rate of oxygen consumption as the test progresses.

Reject such data as being unrepresentative of the suspension oxygen consumption rate, and repeat the test by beginning with higher initial DO levels, for example, by increasing the dilution ratio of tap water volume to sample volume.

vii. If the mixed liquor is diluted to 50% of its original concentration (for a 1 : 1 dilution), the resultant slope of the line should be multiplied by 2 to obtain the *in situ* R value. The time lapse between sample collection and uptake rate measurement is critical in this *ex-situ* procedure. The entire process, from sample collection to the start of DO monitoring, should take less than 2 minutes [9]. The procedure should be replicated at least three times at any given sampling point. The results of this determination are quite sensitive to water temperature variations, and low precision may result unless replicate determinations are made at the same temperature.

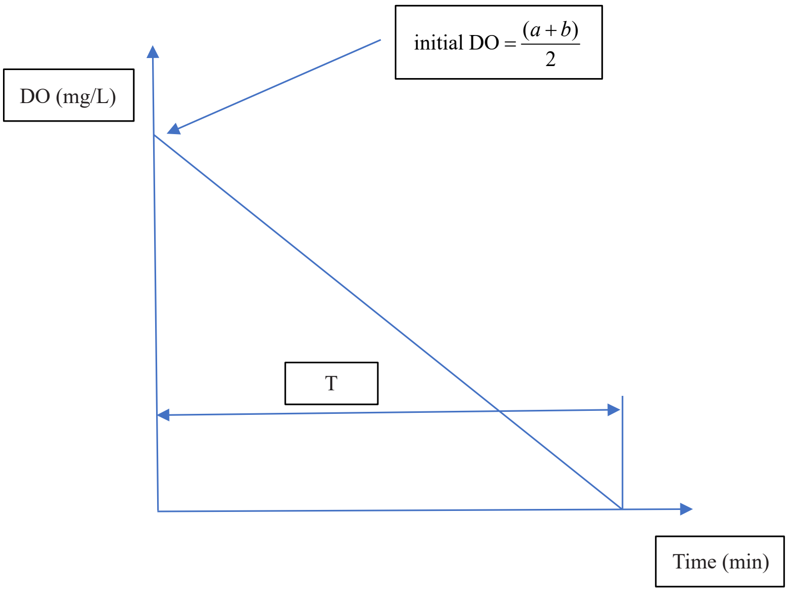

Figure 5. Plot of DO (mg/L) versus time (minutes)

A worked example for a 100% dilution is given below. Suppose the treatment plant *in-situ* aeration tank has a DO level of 2 mg/L under the test conditions, and thus the collected sample should have the same DO concentration at the beginning of the measurement. Next, suppose the bench-scale aeration unit (as shown in Figure 2) has been aerated to 7 mg/L; the mixture will then have a DO concentration of $(a + b)/2 = (2 + 7)/2 = 4.5$ mg/L. If the DO level progressively drops to zero or close to the critical level in 7 minutes, then the slope of the linear decline curve would be $4.5/7 \times 60 = 38.57$ mg/L/hr. The actual oxygen uptake rate *in situ* is therefore given by twice this value, i.e., $2 \times 38.57 = 77$ mg/L/hr. This experiment should then be compared with the BOD bottle method ([2], Method 2710B) to confirm whether the disturbance due to shaking was the real cause of overestimation or if it was caused by increased oxygen availability. By repeating the test for different dilution factors, it can be determined whether a linear-type "different dilutions vs. *R*" relationship is consistently obtained. The initial DO would change according to the different volumetric dilution factors, and statistical procedures can be established for Quality Assurance/Quality Control (QA/QC) purposes to be included in the method statement for the new technique.

3. Results of a dilution test

Note that the dilution factor may affect the outcome of the oxygen uptake rate in the same way that it affects the mass transfer coefficient, leading to variations in the mass transfer coefficient, as shown in a typical example presented in Figure 6.

Here, tap (drinking) water is diluted with samples of mixed liquor feed, mixed liquor effluent, and final clarifier effluent individually at various dilution ratios, yielding the test results shown in Table 1 [8].

Note that even small amounts of dirty water (e.g., 20%) cause a significant reduction in alpha α, ratio of the process water $(K_L a_f)_{20}$ to the clean water $K_L a$ at equivalent conditions, which suggests that either there are substantial concentrations of alpha-reducing substances that consistently depress alpha values in the mixed liquors, or that other mechanisms may be working to reduce the mass transfer coefficient in a respiring setting. In other words, the resulting oxygen consumption rate may not be constant across different dilutions. However, given that there are no chemical reactions involved in the mixed liquor, trial-and-error testing on various samples should determine which dilution ratio yields the most realistic oxygen uptake rate. This method should work in any WWTP and help avoid breaking up the floc and possibly artificially increasing the OUR due to turbulence.

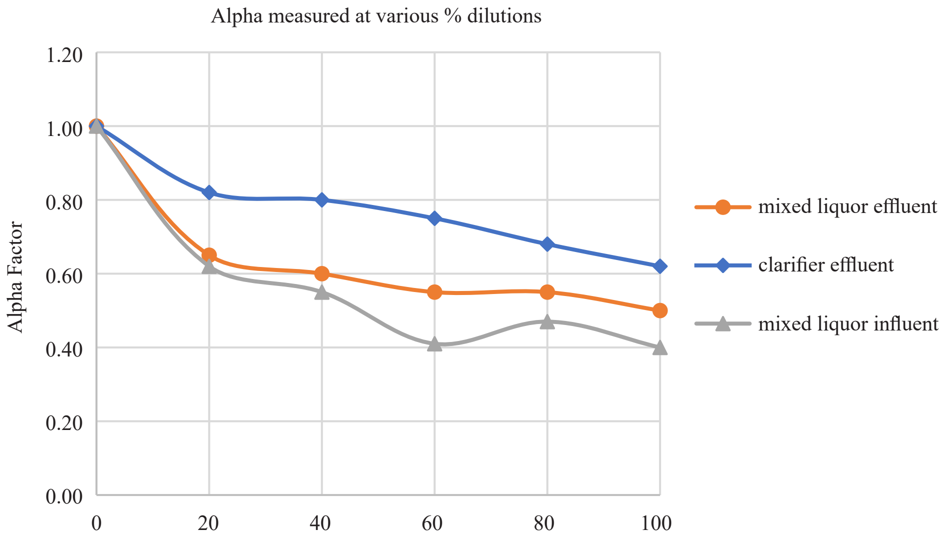

Figure 6. Dilution test results [8]

Table 1. Doyle's dilution test results

dilution %	Alpha measured at various % dilutions		
	clarifier effluent	mixed liquor effluent	mixed liquor influent
0	1.00	1.00	1.00
20	0.82	0.65	0.62
40	0.80	0.60	0.55
60	0.75	0.55	0.41
80	0.68	0.55	0.47
100	0.62	0.50	0.40

There is no reason why it should not provide an accurate measurement of OUR. However, it is worth pointing out that in the case of an oxygen-limited bioreactor (say, operating in the 0-1 mg/L range), it may not allow for the correct measurement of the *in-situ* OUR. Based on the results, and as shown in Figure 1, if one were to take a sample from the low DO region where the OUR is only 40 mV/min, for example, and then dilute it while providing high DO, the measured OUR would be close to 100 mV/min (after making the dilution correction).

4. Effect of erroneous measurement of in-process *R* on calculations of the $SOTR_{pw}$

The sample calculation given in ASCE [9] can be reconstructed as follows, for the schematic of a completely mixed system is shown in Figure 7. The spreadsheet data in Table 2 correspond to Chapter 2, Section 2.5, where a sample calculation of the oxygen transfer rate for a surface aerator is presented, using the test results for Probe No. 2 during the Haverstraw Test No. 8. (Note: The recorder data and calculated nonlinear regression results for Probe No. 2 are plotted in Figure 8, which is similar to Figures 2-5 in [9].) The results of the spreadsheet analysis are given in Table A1.

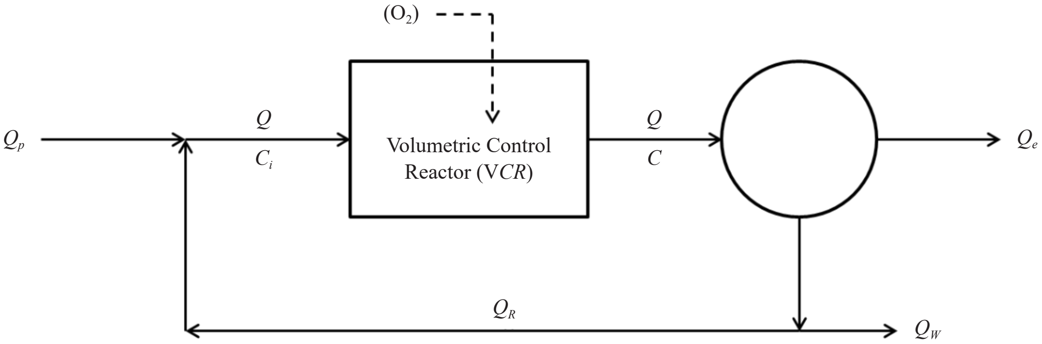

Figure 7. Schematic of a Completely Mixed System ([9], Figure 2-1)

The test was conducted in an aeration tank 3.8 m (12.5 ft) deep, and the measured average oxygen uptake rate was reported as 19.4 mg/L·h. This is a completely mixed basin; therefore, the OUR is 19.4 mg/L·h for the entire basin. As noted in the text, the K_La values calculated from the various probes are remarkably uniform, as would be expected in a completely mixed basin.

Table 2. Nonlinear Estimation for Nonsteady-State Oxygen Transfer: Haverstraw Run No. 8, Probe No. 2 Data

K (1/h)	
4.33534	K, C_R, C_0
K (min^{-1})	0.072256
C_R (mg/L)	3.42313
C_0 (mg/L)	15.17429

The detailed analysis of the collected data is presented in Table A1 and also included in the Appendix. Note that the Oxygen Uptake (OU) values are based on the averaging of multiple measurements (see [9], Figures 2-4). Although the method for measuring OUR was not described in detail, it is presumed to have been conducted using the BOD bottle DO depletion technique ([2], Method 2710B).

Considering that the tank volume is 783 m^3, this will give an oxygen consumption rate of $19.4 \times 783 \times 10^{-3} = 15.2$ kg O_2/h.

The other data collected are as follows:

Q (Total influent) = 9,727 m^3/d [Q_p (Primary Effluent) + Q_R (Return Sludge) = 6,283 m^3/d + 3,444 m^3/d]

mixed liquor temperature = 20 °C

tank volume = 783 m^3

C_i (DO concentration in Q) = 2.3 mg/L

The tank detention time is calculated as:

$$\text{detention time} = V/Q = 783/9{,}727\ \text{m}^3/\text{d} \times 24\ \text{h/d} = 1.93\ \text{h}.$$

From ASCE [9] Eq. (2-5), the calculated average $K_La_f = K$ – (1/detention time) = 4.33 (as shown in Table 2) – (1/1.93) = 3.81/h. Therefore, $(K_La_f)_{20}$ = 3.81/h, since the test was done at 20 °C and the recorder data and calculated nonlinear regression results for Probe No. 2 is as plotted out in Figure 8 which is similarly equivalent to Figures 2-5 in [9].

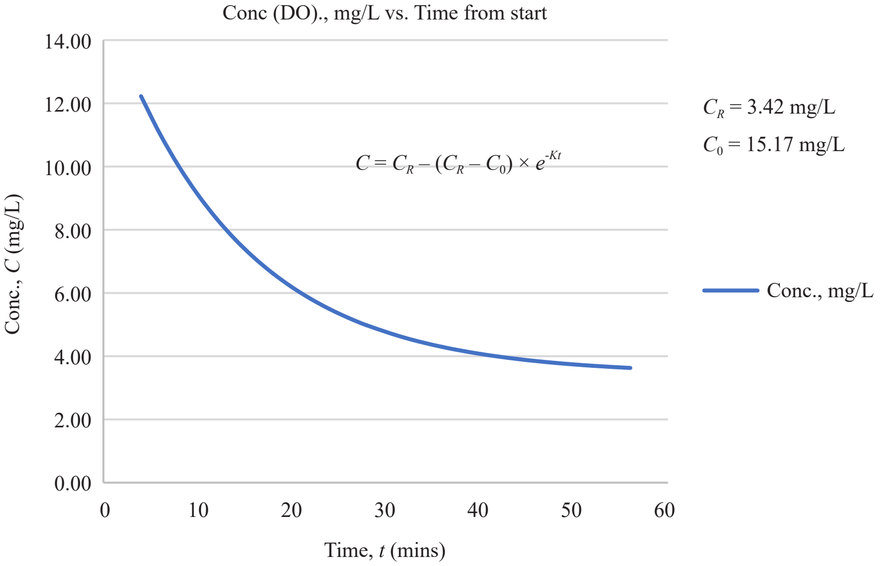

Figure 8. Nonsteady-state oxygen curve based on Non-Linear Least Squares (NLLS) fitting of data ([9], Figures 2-5 (Re-constructed))

As Baillod [11] mentioned that, “Many pieces of equipment nominally referred to as surface aerators cause subsurface entrainment of air with the result that C^* (DO saturation (equilibrium) concentration in the liquid phase) is not constant over the volume and the average value of C^* becomes somewhat greater than the surface saturation value. A sub-surface aeration model based on an effective average saturation value would be more applicable to this situation.”

In ASCE/EWRI Standard 2-22 [12] Chapter C6, assuming a subsurface model Eq. (C6-4) gives C^*_∞ = 9.1 + 0.1 times diffuser submergence, in feet = 9.1 + 0.1 × 12.5 = 10.35 mg/L, therefore, according to ASCE [9], the $SOTR_{pw}$ = (0.99) (10.35) (783) (3.81×10^{-3}) = **30.5** kg O_2/h as opposed to 26.7 kg O_2/h calculated in the ASCE text. This is almost double the consumption rate of 15.2 kg/h, assuming the consumption rate remains the same at near-zero DO level.

Since oxygen depleted in gas must equal oxygen absorbed by liquid (same as the consumption rate in the steady state), there appears to be a substantial overestimation of the SOTR in the process water for the period of the test.

Alternatively, considering a mid-depth correction, treating the aeration as subsurface, the equilibrium pressure would be:

$$P = Ps + 0.5 \times (997\ \text{kg/m}^3 \times 9.81\ \text{N/m}^3/(\text{kg/m}^3)) \times 3.8\ \text{m}.$$

Assuming Ps = 1.013 E5 N/m^2, where Ps is the surface pressure and therefore, the mid-depth pressure gives P = 119,883 N/m^2.

Assuming the mid-depth mole fraction Y_e = 0.19 (again assuming a bubble aeration scenario to mimic gas depletion), we have:

$$C^* = H \times Y_e \times P \text{ (where } H \text{ is Henry's constant } H = 4.3819 \times 10^{-4}\ \text{mg/L/(N/m}^2\text{) at 20 °C)}$$

$$C^{*} = (4.3819 \times 10^{-4})\ (0.19)\ (119{,}883) = 9.98 \text{ say } 10 \text{ mg/L}$$

Using the modified equation proposed by Lee [13], which incorporates the gas-depletion effect, the relationship is expressed as follows:

Modified version of Eq. (2-3) used for the non-steady-state method.

What was proposed by Lee [13] is that the saturation concentration, C^{*}, should be expressed as a function of the oxygen utilization rate, R, or,

$$C^{*} = C_{\infty}^{*} - R/K_L a_f \tag{1}$$

For the transfer equation as given by Magdalena et al. [14]: $dC/dt = K_L a\ (C^{*} - C) - R$, substituting the value of C^{*} into the transfer equation gives

$$dC/dt = K_L a\left(\left(C_{\infty_f}^{*} - R/K_L a_f\right) - C\right) - R \tag{2}$$

When the system is at steady state, $dC/dt = 0$, and $C = C_R$, $K_L a = K_L a_f$ so that

$$K_L a_f = R/\left(C_{\infty_f}^{*} - R/K_L a_f - C_R\right) \tag{3}$$

Expanding the equation gives

$$R = K_L a_f \cdot C_{\infty_f}^{*} - R - K_L a_f \cdot C_R \tag{4}$$

Therefore,

$$2R = K_L a_f \left(C_{\infty_f}^{*} - C_R\right) \tag{5}$$

as opposed to Eq. (2-3) in ASCE [9], which applies only to a batch process and thus gives only

$$R = K_L a_f \left(C_{\infty_f}^{*} - C_R\right) \tag{6}$$

It is important to mention that the $K_L a$ coefficient is an empirical value that must be estimated or experimentally assayed (typically in an abiotic setting). $K_L a$ attribution can introduce considerable error when estimating mass transfer rates in biological systems because biological activity directly affects the properties of the aqueous system through, for example, the release of biosurfactants and other metabolites. However, it is the author's belief that such effects as gas-phase oxygen depletions due to oxygen consumption are predominantly more pronounced on C^{*} than the surfactant effects on the mass transfer coefficient $K_L a$. Therefore, by means of superposition,

$$SOTR_{pw} = \beta \cdot C_{\infty}^{*} \cdot K_L a_f \cdot V - 0.001 RV \tag{7}$$

Therefore,

$$SOTR_{pw} = 0.99 \times 10 \times 3.81 \times 10^{-3} \times 783 - 0.001 \times 19.4 \times 783 = 29.5 - 15.2 = \mathbf{14.3}\ \text{kg/h}$$

the uptake rate is given by RV,

$$RV = 19.4 \times 783 \times 0.001 = 15.2\ \text{kg/h}$$

which is not far from the transfer rate of 14.3 kg/h.

This result appears to be more reasonable, even though the influences of Q_R and Q_p in the flow process have not been included. Therefore, the proposed equation appears to be valid, and the standard guideline result of $SOTR_{pw}$ = 26.7 kg/h is inferred to be a substantial overestimation, even if R is measured correctly.

It is therefore concluded that the conventional equation, which does not take into account Gas-Phase Oxygen Depletion (GDP), results in a significantly under-designed system when clean water data (C^* and implicitly K_La) are used to extrapolate to field conditions without the appropriate relationships with the respiration rate.

On the other hand, it is important to recognize that the calculated "standard" $SOTR_{pw}$ of 30.5 kg/h is taken at 0 mg/L DO. This value represents the maximum transfer rate that could be achieved under 0 mg/L DO conditions, based on the calculated K_La_f and an assumed DO saturation value. However, this does not represent the actual steady-state Oxygen Transfer Rate (OTR) that occurs at higher DO levels. In this case, at steady state, the actual DO is 3.42 mg/L, as obtained from the curve fit. At that steady-state DO, the OTR equals the OUR.

Using the industry standard assumption for surface saturation with a surface aerator, we can calculate a steady state oxygen transfer value, which should equal the OUR.

At steady state, according to ASCE [9] Eq. (2-3),

$$R\ (\text{mg/L/hr}) = (2.3 - 3.42)/1.93 + 3.81 \times (0.99 \times 9.09 - 3.42)$$

$$R = \mathbf{20.7}\ \text{mg/L/hr}$$

where C_s = 9.09 mg/L (based on handbook value at 20 °C).

It can be observed that the measured OUR of 19.4 mg/L·h agrees reasonably well (approximately 6.7% difference) with the calculated value of 20.7 mg/L·h. It is, of course, difficult to perform a truly steady-state test during actual plant operation, since in practice no system is ever in a perfectly steady state. Beyond the issues associated with the measurement of R and α, there is a possibility that K_La may be dependent on the oxygen uptake rate in respiring systems. If so, Eq. (2-3) may be inaccurate, as it does not reflect such a dependency [13].

5. Practical examples for modification of Eq. (2-3)

An example in Garcia et al. [15] showing the in-process model discrepancies is given in the supplemental material. The dynamic measurement of the OUR and K_La is shown in Figure S1. The calculation of C_R is given in Table S1, using the Excel Solver method. Comparisons of the conventional model and the proposed model are illustrated by Eqs. (S1-S4). This example appears to show that the OTR is related to the respiration rate R, indicating that Eq. (1) above applies.

Returning to the current example, for a flow-through system, OTR is not exactly equal to OUR at steady state. One must account for the mass inflow and outflow of oxygen, which Eq. (2-3) does. As Eq. (2-3) states:

$$R = \left(C_i - C\right)/\text{HRT} + \text{OTR} \tag{8}$$

where OTR = $K_La_f(C_s - C_R)$, and only when $C_i = C$ does OTR = R. However, the term $(C_i - C)$/HRT is small compared with $K_La_f \times (C_s - C_R)$, giving 0.58 vs. 26.00 for the two terms.

The calculated OUR presented above already includes the oxygen mass balance term, $(C_i - C_R)$/HRT. The value of R obtained using the non-steady-state analysis agrees very well with the measured R.

Consider the mass basis, the oxygen consumption mass rate can be expressed as:

Based on the measured R value: OUR = 19.4 mg/L/hr × 783 m^3/1,000 = 15.2 kg/hr

Based on Eq. (2-3): OUR = 20.7 mg/L/hr × 783 m^3/1,000 = 16.2 kg/hr

The above calculation seems to have justified Eq. (2-3) and so it would seem that GDP is not significant in an open-air surface aeration system. Certainly, it would be difficult to measure for surface aeration.

The fact that Eq. (2-3) happens to produce the same R as that measured by the BOD bottle method is likely a coincidence, further complicated by the assumed saturation concentration of 9.09 mg/L, which is not fully justified.

6. Result based on the revised Eq. (2-3)

With the proposed modifications by Lee [13] and Lee [16], Eq. (2-3) becomes

$$2R = \left(C_i - C_R\right)/\mathrm{HRT} + K_L a_f \left(C^*_{\infty f} - C_R\right) \tag{9}$$

i.e.,

$$2R = (2.3 - 3.42)/1.93 + 3.81(10.35 \times 0.99 - 3.42)$$

[Note that $C^*_{\infty f}$ is not 9.09 mg/L but 10.35 mg/.]

Thus,

$$-0.58 + 26.00 = 2R \Rightarrow R = 25.4/2 = \mathbf{12.7}\ \mathrm{mg{\cdot}L^{-1}{\cdot}h^{-1}}$$

This shows that the BOD bottle method yields an overestimated R, and the value of 19.4 mg/L·h is not accurate if the modified equation is valid.

The true $SOTR_{pw}$ at C = 0 mg/L, assuming that R has not changed, should be approximately:

$$12.7 \times 783 \times 10^{-3} = \mathbf{9.95}\ \mathrm{kg/h} << 30.5$$

This value is much lower than the previously calculated 30.5 kg/h, highlighting a significant discrepancy between the two models.

The GDP term, which is equivalent to R, is missed out in the original equation, giving an over-inflated R value (20.7 vs. 12.7 mg·L^{-1}·h^{-1}) as much as almost twice its true value. This is very unfortunate, as the ASCE-calculated value of 26.7 kg/h cannot match up with any calculated R consumption rate, and the discrepancy will only increase with higher treatment rates (i.e., higher OUR). The greater the biochemical activity in the basin at a fixed gas flow rate, the lower the $SOTR_{pw}$, owing to the gas depletion effect, which has been verified in numerous studies by López et al. [17], García-Ochoa et al. [15], and others.

Of course, $SOTR_{pw}$ corrected to 0 mg/L DO is higher than 15.2 or 16.2 kg/hr. As the operating DO increases, the OTR decreases as the driving force decreases. The true driving force at steady state is 0.99 × 9.09 – 3.42 = 5.58 mg/L. The calculated $SOTR_{pw}$ does not represent the actual oxygen transfer rate at steady state. The calculation at 0 mg/L uses a driving force of 0.99 × 9.09 = 9 mg/L. As a result, one gets a corrected to 0 mg/L OTR value of 9/5.58 times the actual OTR at the steady state DO.

Therefore, if the ASCE equation (Eq. (2-3)) is deemed correct giving an OUR of 16.2, then

16.2 × 9/5.58 = 26.1 kg/hr – at 0 mg/L DO.

This is fundamental if the original equation is correct.

At steady state, OTR = OUR (or adjusted slightly according to the mass flow in or out of the tank). Comparing the conventional and modified methods, as shown in Table 3, the true Driving Force (DF) at steady state is ($C^* – C_R$) but C^* is given by:

$$C^* = C^*_{\infty f} - R/K_L a_f \tag{10}$$

Therefore,

$$C^* = 10.0 \times 0.99 – 12.7/3.79 = 6.55 \text{ mg/L}$$

Table 3. Comparison of the two models

	conventional method	modified Eq. (2-3)
OUR (APHA)	19.4/20.7 $\text{mg}\cdot\text{L}^{-1}\cdot\text{h}^{-1}$	-
OUR (dilution)	-	12.7 $\text{mg}\cdot\text{L}^{-1}\cdot\text{h}^{-1}$
$SOTR_{pw}$ ($R = 0$)	26.7 kg/h	30.5 kg/h
OTR (SS)	16.2 kg/h	9.95 kg/h
True DF	5.58 mg/L	3.13 mg/L
DF ($C = 0$)	9.0 mg/L	9.9 mg/L
correction	1.6	3.16
$SOTR_{pw}$ (calc.)	1.6 × 16.2 = 26 kg/h	3.16 × 9.9 = 31.3 kg/h

SS: Steady-State

Therefore, DF (SS) = 6.55 – 3.42 = 3.13 mg/L.

The discrepancy in the new method between the measured and the calculated $SOTR_{pw}$ is in the assumed mole fraction of the exit gas of 0.19 which is reasonable but is a complete guesswork. But the discrepancies in the conventional method are, first of all, the measurement of $R = 19.4 \text{ mg}\cdot\text{L}^{-1}\cdot\text{h}^{-1}$, which is incorrect, due to the inherent shortfall of the BOD bottle technique that artificially gives a higher value than the true value, is more realistically given by the modified Eq. (2-3), for the result of 12.7 $\text{mg}\cdot\text{L}^{-1}\cdot\text{h}^{-1}$ instead of 19.4 $\text{mg}\cdot\text{L}^{-1}\cdot\text{h}^{-1}$; and secondly, the incorrect driving force at the steady state results in an erroneously high OTR under test conditions. Although the calculated value using Eq. (2-3) seems to match the measured value, both are incorrect, and the end result would be a K_La value would be underestimated if the conventional equation and measured R were used. This observation is consistent with data in ASCE [9] Table 5-2, which compares K_La_f values obtained using the SS and Non-Steady-State (NSS) methods for various on-site measurements.

7. Controversy and discussion

The controversial issue is that $C^*_{\infty f}$ is substantially higher than 9.09 mg/L, even in a surface aeration system. When clean water tests are conducted with Orbal disc aerators [5], the clean water saturation is very close to the surface saturation value at that temperature, and any significant increase in C_s due to subsurface entrainment is not observed with that particular aeration device. In a diffused air system, one will see the increase due to depth of the air release. Perhaps, in some other surface aeration systems which inject bubbles below the surface, there is an entrainment effect.

Over years of testing in respiring systems, it has been found that the standard ASCE equation matches up with data quite well for the Orbal aeration system. Moreover, no consistent findings in literature suggest that there should be a $2R$ term in the mass balance equation in a respiring system. However, the actual errors could be a combination of the various effects, paramount of which is the GDP. To fully convince the scientific community, it will be necessary to demonstrate experimentally whether GDP indeed exists in these systems through carefully controlled studies.

However, it is worth remembering that the steady-state saturation concentration in clean water is not the equilibrium concentration (C^*) in in-process water. In a study by Mahendraker et al. [18], the biological floc mass transfer coefficient (K_La_{bf}) was estimated from the mass transfer coefficient of the mixed-liquor (K_La_f) and the reactor-solution (K_La_e). The Biological Floc Resistance (BFR) and Solution Resistance (SR) were defined as the reciprocals of K_La_{bf} and K_La_e, respectively, by applying the serial-resistance concept originally presented in two-film theory Lewis and Whitman [19]. The Specific Biological Floc Resistance (SBFR) was defined as the biological floc resistance per unit biomass concentration. The data indicated that activated sludge systems yielding low BFR/MLR and BFR/SR ratios tend to produce higher oxygen transfer efficiency. Surprisingly, the reactor-solution posed the same level of resistance as clean water in all experiments, except in a 5-day SRT, non-nitrifying, Completely Mixed Activated Sludge (CMAS) process. Furthermore, SBFR successfully represented biological floc and showed a positive correlation to Sludge Volume Index (SVI). In addition, the SBFR/SR ratio and Oxygen Transfer Efficiency (OTE_f) followed an exponential relationship for the complete data set. The method of separating the mixed-liquor into biological floc and reactor-solution improved the understanding of oxygen transfer under process conditions, without resorting to intrusive techniques or direct handling of fragile biological floc. This study by Mahendraker unequivocally showed that the biological floc has an impact on the oxygen transfer rate and the data has been thoroughly investigated by Lee [16]. The re-investigation confirmed that the oxygen transfer rate is affected by the microbial respiration rate, which would result in a modification of the ASCE [9] Eq. (2-3) giving a $2R$ term instead of a single R term.

Hines et al. [20] and Boon [21] have claimed that the maximum rate at which oxygen would be required by high concentrations of microorganisms, treating a readily biodegradable organic substrate under ideal conditions in a fermentation unit, is approximately 10 kg $O_2/m^3 \cdot h$. However, from an activated sludge plant, it is generally necessary to produce an effluent of low BOD (< 20 mg/L); the rate of biochemical oxidation is therefore limited by available substrate and the concentration of microorganisms, and by the capacity to settle and recycle the sludge. Data indicate that, when the BOD of the effluent was 20 mg/L, the rate of biochemical oxidation was only 1 g/g·day. To dissolve oxygen at that rate would require an aeration intensity of only 0.2 kg $O_2/m^3 \cdot hr$, assuming that the concentration of mixed liquor suspended solids was 5,000 mg/L and no additional oxygen was required for nitrification. This intensity of aeration was easily achieved in the laboratory with a fine-bubble diffused air system, passing air at a high rate through closely spaced diffusers at the bottom of a shallow (0.6 m) tank, and frequently cleaning the external surfaces of the diffusers. The maximum intensity normally achieved with full-scale conventional aerators is about 0.1 $kg/m^3 \cdot hr$ or an R value of 100 mg/L·h, and this may limit the maximum rate of treatment. In wastewater treatment, this will be considered high rate, and so the DO should not be required to reach such a low level (< 2 mg/L) to effect treatment. There would be a risk of plant failure if the operators cut it too fine. Normally, they control the plant to a DO level of 2.5 mg/L, and that's almost regardless of the load coming in or what effluent quality one is achieving, or what one wants to achieve. That tends to give good compliance performance, so most operators are purely reactive to the DO set point. The concept is that if the DO level drops below a set point, then a control system will increase the blower speed or open another valve to increase air supply to the system.

A departure from this approach is beginning to emerge nowadays, with numerous companies offering alternative–and potentially more sophisticated–solutions that aim to help utilities improve their aeration efficiency without jeopardising discharge compliance. It is recommended to save energy by reducing the amount of oxygen given to the aeration tank. For example, reducing a dissolved oxygen set point from 2.5 mg/L to 2.2 mg/L can actually generate up to 20% energy savings. Supplying only the oxygen actually required to maintain process stability allows for more efficient and biologically favorable conditions. But this is not the same as cutting the DO level to such an extent as to make the respiration endogenous, i.e., in the neighbourhood of 0.1~1 mg/L. Nevertheless, reducing the DO set point will increasingly make it more difficult to measure the *in-situ* oxygen uptake rate for the microbial respiration. Therefore, it is critical to avoid disturbing the actual DO concentration in a sample prior to measurement to prevent the possibility of over-estimation.

The steady-state *R* technique attempts to measure the *in-situ R* in a full-scale aeration tank and use that measurement, combined with aeration airflow, to determine oxygen transfer parameters. Unfortunately, the measurement of *in-situ* uptake rate is considered extremely difficult when a mixed liquor sample is withdrawn from a tank and the uptake rate is measured in an *ex-situ* device. When oxygen-limiting conditions exist in the sample volume, a significant overestimate of *R* is made when oxygen-starved organisms are provided high-oxygen concentrations during uptake analysis, as stated in ASCE/EWRI 18-18 Appendix D [9].

8. Conclusion

This paper presents an argument that the inaccurate measurement may be due to the agitation of the sample liquid that shears the biofloc and exposes the microbes to higher respiration rates, and offers a solution for accurate measurement. As well, the microbial stress due to the agitation may in turn increase the respiration rate, which is undesirable.

To extricate the experiment from this predicament, it is recommended that the sample be subjected to pure water dilution (or tap water dilution) to produce a high DO content without agitation, in contrast to the conventional method stipulated by APHA [2]. Furthermore, with the modified equation for oxygen transfer replacing Eq. (2-3) in ASCE [9] as proposed by Lee [13], it is expected that the more accurate measurement as described in the dilution method should match the calculated result obtained from the modified equation for the Actual Oxygen Requirement (AOR), from which the Standard Oxygen Requirement (SOR) can be calculated.

Once we have an SOR, aeration equipment that meets that SOR can be selected. The designs for surface aerators are based on a certain kg/hr of oxygen under standard conditions (20 °C, 1 atm, 0 mg/L DO). In this case, 26.7 kg/hr is not truly a "standard" condition because the K_La was determined in dirty water in the presence of microbial respiration, and is not based on a clean water mass transfer coefficient, although the DO is 0 and temperature is 20 °C, which are indeed already at standard conditions. There must be a design alpha value (α) applied to calculate a clean-water K_La. With surface aeration, that alpha factor is usually close to 1 for domestic sewage, typically taken as about 0.9-0.95. According to Doyle [5], this design protocol in most cases works quite well. Plants meet the treatment design loads and produce high quality effluent consistent with effluent standards. However, this good result is a false sense of solace, as it is based on two fallacies of the procedure, as explained in section 5. These fallacies appear to compensate each other, thereby masking the major error in the OUR measurement. The detailed analyses of a surface aerator as given in the example and the subsequent calculations, and a diffused aerator, as shown in the supplemental material appear to validate Eq. (5), (7), and (10).

Therefore, it may be advisable to repeat this ASCE experiment, and to confirm that the true respiration rate, *R*, is 12.7 mg/L·h and not 19.4 mg/L·h, as stated. The preferred method to measure *R* accurately is described in section 2 and section 2.1. Furthermore, the $SOTR_{pw}$ of 30.5 kg/h would replace 26.7 kg/h, as given in the ASCE [9] estimation at zero DO and when a steady state of $R = 19.4$ mg/L·h (this value is widely suspected to be incorrect) is attainable; but the true $SOTR_{pw}$ should be given by $12.7 \times 783 \times 10^{-3} = 9.95$ kg/h under test conditions, considering that this parameter is actually dependent on *R* at zero DO, which in this case is assumed to be unchanged. At $R = 12.7$ mg/L·h under test conditions only, the $SOTR_{pw}$ would be calculated to be 31.3 kg/h and not 26.7 kg/h, when $R = 0$ and DO = 0. Much depends on whether $SOTR_{pw}$ is defined at $C = 0$ and $R = 0$, or whether it should be at $C = 0$ and *R* the same as per the steady-state test. This understanding would qualify the contents of Table 3. For the selection of aeration equipment, the $SOTR_{pw}$ can only be specified as clean water performance, even though in some countries the water is tested with detergent added to roughly simulate oxygen transfer in wastewaters.

According to ASCE [9], additional discrepancies between *in-situ R* and *ex-situ* estimates happen when the *ex-situ* R-measurement device increases the DO of the withdrawn sample compared to the mixed liquor DO at the sample location (and vice versa). This is especially true for nitrifying aeration systems, where nitrification rates increase substantially between DO levels of 0.5 and 2.0 mg/L. Using a previously developed biological model (not stated) [9], withdrawing a nitrifying mixed liquor from an aeration tank with a DO of 0.5 mg/L and raising its DO to 2 mg/L in the *ex-situ* device results in a 50% increase in nitrogenous OUR in the *ex-situ* device.

To alleviate the many problems associated with measuring the biological uptake rate, the proposed method using dilution with saturated DO should give a more accurate measurement than the current standard method using a sample shaking technique as described in APHA [2]. With a more accurate measurement of the OUR, it may lend credence to the modification of the fundamental oxygen transfer equation proposed by Lee [13] for a respiring system, as applied to an example provided by ASCE/EWRI 18-18 [9] that was recently published.

Data availability statement

All data, models, and code generated or used during the study appear in the published article.

Conflict of interest

The author is not affiliated with or involved with any organizations or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this paper.

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Appendix A

Table A1. Nonlinear Estimation for Nonsteady-State Oxygen Transfer: Haverstraw Run No. 8, Probe No. 2 Data (Note that p. conc. is the predicted concentration; delta is the error difference between model predictions and the measured values)

Time (min)	Conc., mg/L	Fit Value	Residual	p. conc.	delta	$(\text{delta})^2$
4	12.176	12.2247	-0.0486956	12.22	-0.05	0.002
6	11.008	11.0404	-0.0324011	11.04	-0.03	0.001
8	10.144	10.0155	0.12854	10.02	0.13	0.017
10	9.104	9.12843	-0.0244293	9.13	-0.02	0.001
12	8.32	8.36076	-0.0407534	8.36	-0.04	0.002
14	7.792	7.69637	0.0956278	7.70	0.10	0.009
16	7.072	7.12139	-0.04386	7.12	-0.05	0.002
18	6.624	6.62377	2.32E-04	6.62	0.00	0.000
20	6.24	6.19311	0.0468936	6.19	0.05	0.002
22	5.76	5.82039	-0.0603924	5.82	-0.06	0.004
24	5.52	5.49783	0.02217515	5.50	0.02	0.000
26	5.248	5.21867	0.0293322	5.22	0.03	0.001
28	4.96	4.97707	-0.0170693	4.98	-0.02	0.000
32	4.56	4.58702	-0.0270233	4.59	-0.03	0.001
36	4.256	4.29488	-0.0388803	4.29	-0.04	0.002
40	3.952	4.07607	-0.124066	4.08	-0.12	0.015
44	3.872	3.91218	-0.0401764	3.91	-0.04	0.002
48	3.84	3.78942	0.0505767	3.79	0.05	0.003
52	3.776	3.69748	0.0785182	3.70	0.08	0.006
56	3.68	3.62862	0.0513816	3.63	0.05	0.003
					$\sum =$	0.072

Appendix B

In the dynamic method example, the airflow inlet to the fermentation broth is interrupted for a few minutes so that a decrease of DO concentration can be observed (Figure S1). When the DO has dropped to an acceptable level, air is turned back on under the same operational conditions until it reaches the same steady state as before. The OUR is determined from the decay-curve slope after the cessation of airflow, and the procedure is repeated several times for precision. In their tests, R is measured *in situ*, and both R and C_R are measured. The second part of the dynamic method is actually identical to the oxygen profile data method that Garcia described, in that both methods require generating an oxygen profile curve (Figure S1). In the dynamic method, the re-aeration is made following the de-aeration by the microbial consumption after stopping the air supply (This re-aeration curve allows the K_La to be calculated). However, the dynamic method requires the OUR to be separately determined, as mentioned in the first part of the test, to be substituted into the oxygenation curve equation to determine K_La. Contrasting with the profile data method where the K_La is pre-determined by other means, including the re-aeration curve, the OUR can be calculated directly from the basic oxygen transfer equation, similar to ASCE Guidelines' equation (2-3) or Eq. (S1). Therefore, in Garcia's example, since the oxygenation profile is created by re-aerating back to the original DO level, which is similar to the ASCE non-steady state method, which is similar to the profile method, the OUR so determined should theoretically be the same as the dynamic method in this example. However, it is not (See calculations below). The calculation shows that the two R values differ by 50% when comparing the uptake test with the re-aeration test.

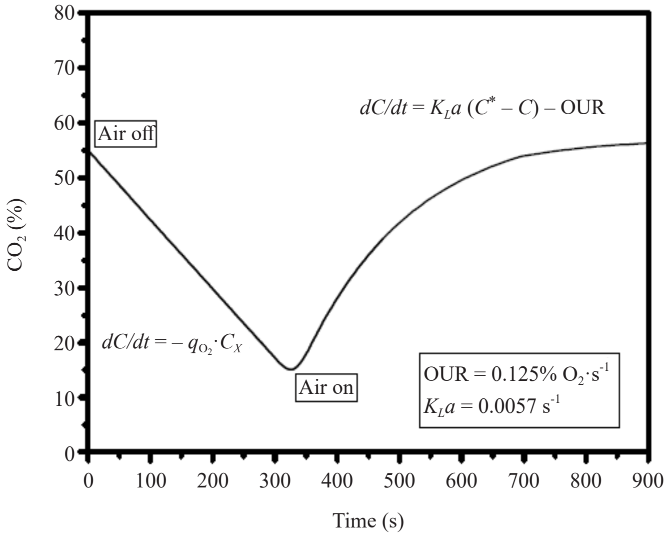

Figure S1. Dynamic measurement of OUR and K_La

Garcia's equation is identical to the ASCE equation given as: (Note that C^* in his equation is used in the context of C^*_∞)

$$dC/dt = K_La\,(C^* - C) - \mathrm{OUR} \tag{S1}$$

$$C = C_R - (C_R - C_0)\exp(-K_La \cdot t) \tag{S2}$$

From Figure S1, it can be read the following: $C_R = 59.5\%$; $C_0 = 14.7\%$, therefore, substituting the readings into Eq. (S2), we have

$$C \approx 59.5 - (59.5 - 14.7) \exp(-0.0057 \cdot t)$$

where K_La is found to be 0.0057 s^{-1} by fitting the read data to the model by the Non-Linear Least Square (NLLS) method, using the Excel solver. Garcia's own calculation in their report gave: $K_La = 0.0057$ s^{-1}. Using Eq. (S1), if the steady state concentration ($C = C_R$) is taken to be around 55%, then R would be calculated as:

$$R = 0.0057 \cdot (100 - 55)/100 = 0.256\%\ s^{-1}$$

However, from the oxygen uptake rate test, using the linear decay curve with the bottom DO concentration of around 13% over a time period of 335 s, we have

$$dC/dt = -R \tag{S3}$$

Therefore,

$$R \approx (55 - 13)/335 \times 100 = 0.125\%\ s^{-1},$$

which is only half the value calculated by the profile method. If this measured uptake rate is inserted in the above equation as a known quantity, the value of K_La obtained is only one-half of that obtained by the NLLS method (the profile method), and will not be correct, since Garcia's formula did not include the effect of microbial resistance. With the inclusion of such an effect, Eq. (S1) should be replaced by:

$$dC/dt = K_1 (K_2 - C) - r - R$$

when the resistance r is equated to R, K_1 is replaced by K_La_f and K_2 replaced by $C^*_{\infty f}$

The equation becomes:

$$dC/dt = K_La_f (C^*_{\infty f} - C) - 2R \tag{S4}$$

where R equates to OUR in Eq. (S1). In this case, when at steady-state, R is measured *in-situ* as 0.125% and as calculated by Eq. (S3) above. Since, at steady-state, $dC/dt = 0$, and C is around 55%, therefore, using the modified Eq. (S1) (now Eq. (S4)) gives:

$$K_La_f = 2 \times 0.125/(100 - 55) = 0.0056\ s^{-1}$$

which is essentially identical to the value measured using the conventional equation employed by Garcia et al. [22] in the profile method (equivalent to the NSS method), which would give $R = 0.256\%$ s^{-1}—twice the value measured *in-situ* by the linear decay curve.

Garcia has done many other similar tests, using different microorganisms, different stirring speeds, and different air flowrates, such as shown in Figure S2 and Figure S3 above. The 2-fold phenomenon is illustrated in all cases.

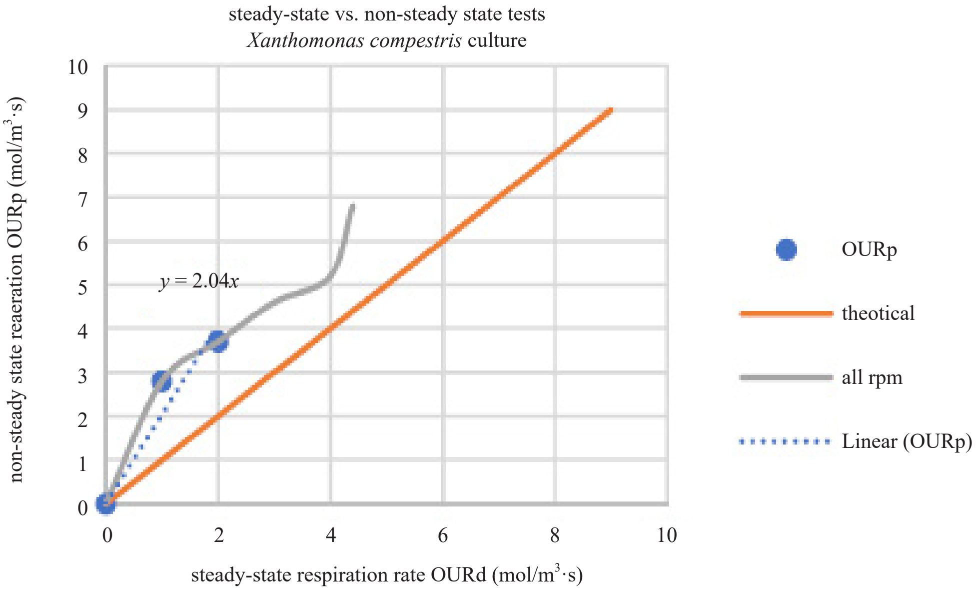

Figure S2. OUR_p vs. OUR_d (mol $O_2/m^3 \cdot s \times 10^{-4}$) [Xanthomonas compestris culture]

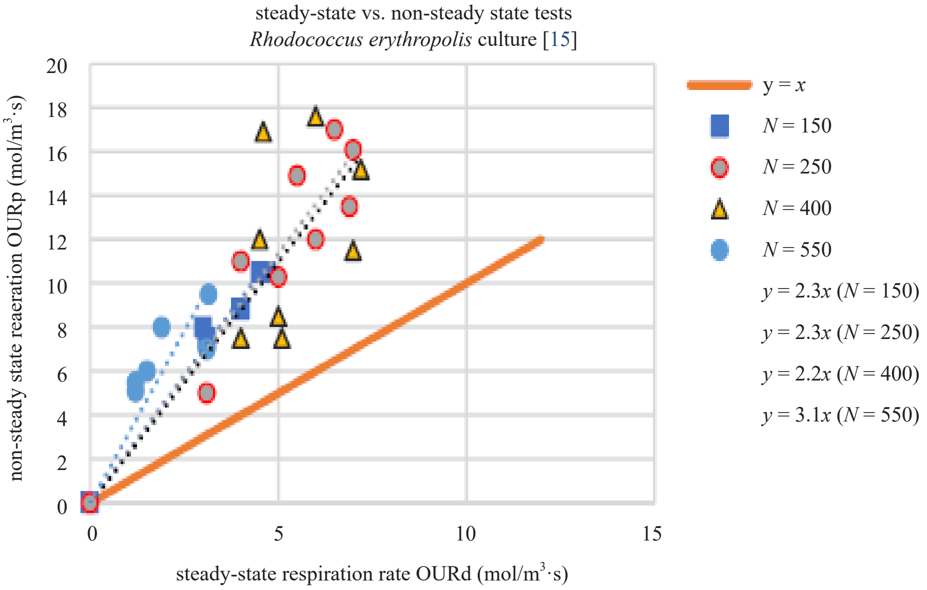

Figure S3. OUR_p vs. OUR_d (mol $O_2/m^3 \cdot s \times 10^{-4}$) [Rhodococcus erythropolis culture]

Table S1. Re-constructed data from [15]

start time	time from start (s)	duration (s)	c (%)	Cr, $K_L a_f$, C_0	c (model)	(c-c (model))	Sum of Sqs.
330	330	0	15	59.49205	14.68	0.322	0.103
	420	90	30	0.005168	31.35	-1.347	1.815
	480	150	40	14.67843	38.85	1.149	1.319
	630	300	50		49.99	0.015	0.000
	900	570	57		57.14	-0.137	0.019
						min. sum (SS)	3.257