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An accurate measurement technique for the biological oxygen uptake rate Date: September 20, 2024

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Abstract

For any wastewater treatment aeration tank, the paper presents an interesting technique to deal with oxygen uptake rate (OUR) measurements. 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 uptake rate only. It would be fascinating to try the proposed dilution vs. the "shake it up" aeration approach to avoid shearing the floc which may increase the OUR artificially, 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 is also dependent on the oxygen concentration in the liquid.

Oxygen uptake rate and dissolved oxygen (DO) are inversely proportional to each other. 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 O2/L/hour), it is a useful tool to evaluate process performance, aeration equipment, and biodegradability of the waste. The oxygen uptake rate is one of the fundamental physiological characteristics of culture growth and has been used for optimizing the fermentation process, and so it needs to be measured accurately. Oxygen uptake rate (OUR) is the microorganism oxygen consumption per unit time and is one of the few accessible parameters to quantify the metabolism rate of the activated sludge in a wastewater treatment plant.

The manuscript observes that if a sample of mixed liquor is withdrawn from an aeration tank operating at low DO, the OUR measured in the sample after shaking (or other means of perturbation) will be higher than the true oxygen uptake rate (OUR) which is limited by the aeration supply. Once a high DO is provided, then oxygen is no longer a limiting factor and the OUR will increase. With sufficient DO the OUR will become a function of the substrate concentration and the biomass concentration in the collected sample for measurement. Also, it is possible that the point at which the 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 this document, one may find a different point at which the OUR starts to drop off (as the DO goes from close to saturation to lower levels).

To alleviate the many problems of measurement, the proposed method using dilution with saturated DO may give a more accurate measurement than the current standard method using a sample shaking technique as described in [1]. With a more accurate measurement of the OUR, it may lend credence to justification for the modification of the fundamental equation for oxygen transfer in a respiring system, as applied to an example provided by ASCE/EWRI 18-18 recently published.

Introduction

One problem with the biological oxygen demand (BOD) bottle type of oxygen uptake rate (OUR) measurement (APHA 2017 method 2710B) is that it can only be used in a system that is not dissolved oxygen (DO) limited (typically assumed 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. That measured OUR will be erroneously high.

The oxygen uptake rate is not solely dependent on dissolved oxygen levels, but rate of oxygen consumption is affected by the DO levels. When the level of dissolved oxygen in the medium falls below a certain point, the specific rate of oxygen uptake is also dependent on the oxygen concentration in the liquid. Oxygen uptake rate and DO are inversely proportional to each other. 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 O2/L/hour), it is a useful tool to evaluate process performance, aeration equipment, and biodegradability of the waste. The oxygen uptake rate is one of the fundamental physiological characteristics of culture growth and has been used for optimizing the fermentation process. Oxygen uptake rate (OUR) is the microorganism oxygen consumption per unit time and is one of the few accessible parameters to quantify the metabolism rate of the activated sludge [2]. and so, it is vital that it be measured correctly.

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 WWTPs (Waste Water Treatment Plants), 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 MLSS (mixed liquor suspended solids), 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 depends on the aeration rate relative to oxygen uptake rate. 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. In a certain sense, the two factors (OUR vs. DO) are inversely proportional to one another. In the method recommended is the BOD bottle method, which is a standard method to determine the R value of a sample of activated sludge [1].

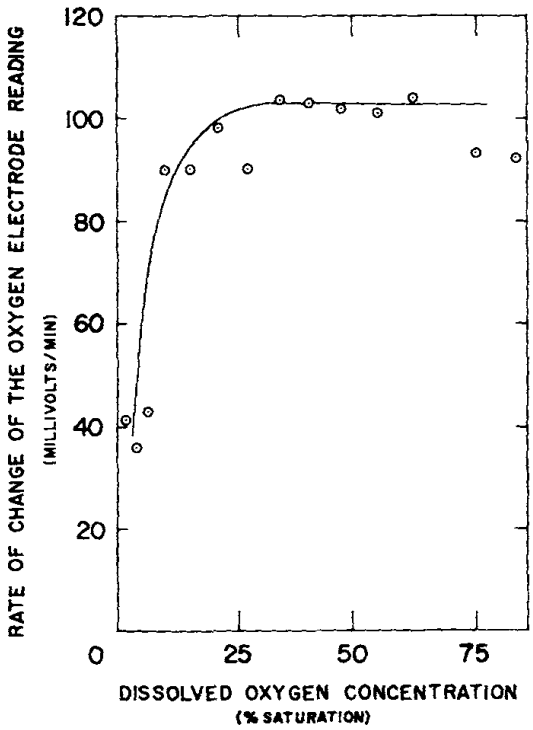

Figure 1: Effect of dissolved oxygen concentration on the rate of oxygen utilization by resting cells of Pseudomonas ovalis with no aeration at pH 7.0 and 25°C [3].

Problem Statement

This APHA method has a fatal drawback, in that it can severely over-estimate the consumption rate when the sample must be aerated to give a linear straight line of a plot for the DO vs. % saturation. Looking at Figure 1 [3]. on the rate of oxygen utilization for the resting cells of Pseudomonas ovalis, one can see that 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 change of the oxygen electrode reading becomes 100 (i.e., the rate of change of R is zero). R becomes a constant until the sample reaches full saturation.

Conversely, when the sample begins with a high DO concentration, say 5 mg/L, R would remain 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 the initial DO must not be artificially aerated by shaking or injecting DO molecules like in a respirometer. Unfortunately, this is the recommendation by the Standard Methods [1]. to increase the initial DO level of the sample prior to measuring the DO rate of decline when the sample only has miniscule DO because the DO level is low in the aeration basin from where 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 [4]. indicated that many factors affect the oxygen utilization rate, R, among which are high concentrations of living organisms and high turbulence levels, not to mention the level of organic substrates present in the sample. High turbulence should have the same effect as shaking or other types of perturbations in a sample bottle, leading to not just a higher DO but also a higher R value. The question is, is the higher R value a consequence of the higher DO given a constant organic substrate level and a fixed number of microbes, or is it due to some other factors such as agitation and excitation of the organisms from shaking the sample causing a higher respiration rate, during the attempt to increase the DO, as stipulated in [1].

Proposed Dilution Method

If it were the latter, the remedy could be just mixing 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 give a decent linearity that 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 "insitu" 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 [1]. stipulates but only by mixing two fluids together to obtain a uniform DO concentration.

In the article by [5]. an interesting method of testing for alpha was suggested. It appears that it may be possible to use the dilution method as suggested by the article to test out the determination. By first aerating a tank of pure water to an elevated DO, say to 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 then gently mixing them together, it may be possible to measure the slope of the DO decline curve at quiescent conditions, thereby eliminating the first possible explanation for the cause of 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 should then be compared with the steady-state column test with an in-situ measurement as recommended by the ASCE/EWRI 18-18 Guidelines (ASCE 2018).

Alternatively, mixed liquor can be continuously pumped to 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 measurement of the slope of the curve as a measurement of the microbial oxygen uptake rate. To avoid any substrate limiting effect, the test should be done in-situ as quickly as possible just like the off-gas column test.

Proposed Steps for the Dilution Method

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

Figure 2: A Typical Bench-Scale Aeration Unit (Image from Armfield Ltd.)

Collect a sample of mixed liquor to be evaluated into a container of approximately 4 to 6 L, as shown in Figure 3 [6].

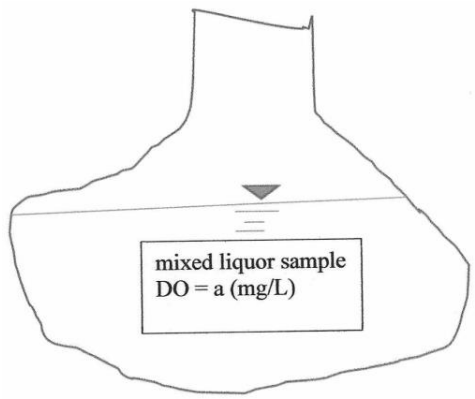

Figure 3: Sample of Mixed Liquor of a Known Volume in a Container

Upon stopping the aeration, gradually and carefully pour the sample of known volume of the activated sludge mixed liquor into the tank as shown in Figure 4 [6]. The bespoke aeration unit as shown in Figure 2 should come with a calibrated fast-response DO probe and a stirring mechanism.

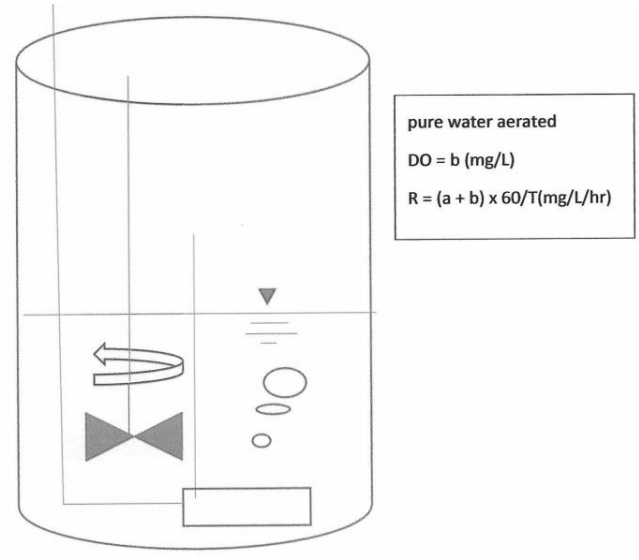

Figure 4: Aeration Tank of Same Volume of Pure Water

Then gently mix them together by the mechanical stirrer until mutual dilution is complete as shown in (Figure 4) [6]. Immediately measure the DO by the Winkler Method or by using the calibrated fast-response DO probe with probe time constants less than 0.02 / KLa. The timing device is started after meter reading has stabilized. Record appropriate DO data at time intervals of less than 1 min, depending on rate of consumption. Record data over a 15-min period or until DO becomes limiting, whichever occurs first.

Plot the DO versus time (T) on a graph similar to that shown in (Figure 5) [6]. and calculate the slope using a linear least-squares regression to fit a straight line through the data points. The oxygen probe may not be accurate below 1 mg/L of DO. Low DO (< 2mg/L at the start of the test) may limit oxygen uptake by the biological suspension and will be indicated by a decreasing rate of oxygen consumption as the test progresses. Reject such data as being unrepresentative of suspension oxygen consumption rate and repeat test beginning with higher initial DO levels, by increasing the tap water volume/sample volume dilution ratio.

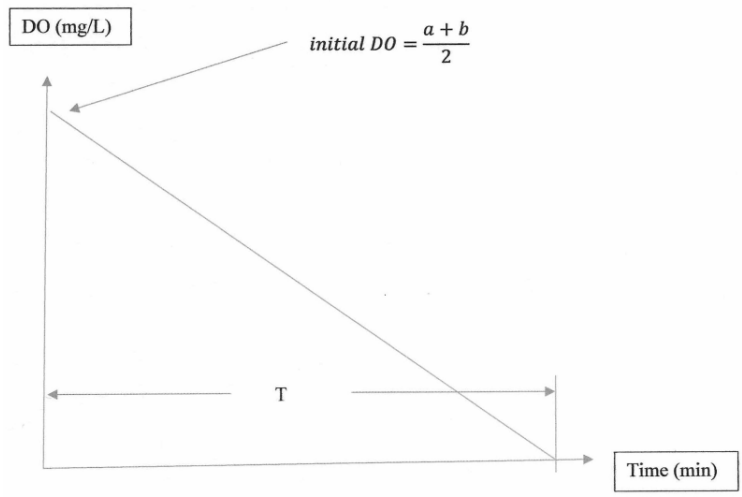

Figure 5: Plot of DO (MG/L) Versus Time (Minutes)

If the mixed liquor is diluted to 50% its original concentration (for a 1:1 dilution), the resultant slope of the line would 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 the collection of samples to starting DO monitoring should take less than 2 minutes [ASCE 2018]. 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 poor precision is obtained unless replicate determinations are made at the same temperature.

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 at the test conditions, and so the collected sample should have the same level of concentration at the beginning of the measurement. Then suppose the bench-scale aeration unit (as shown in Fig. 2) is aerated to 7 mg/L, the mixture will have a DO concentration of: (a + b)/2 = (2 + 7)/2 = 4.5 mg/L Suppose the DO level progressively would have dropped to zero or close to the critical level in 7 mins, then the slope of the linear decline curve would be: 4.5/7 X 60 = 38.57 mg/L/hr The actual oxygen uptake rate in-situ is therefore given by twice this value or 2 X 38.57 = 77 mg/L/hr.

This experiment should then be compared with the BOD bottle method (APHA 2017 method 2710B) to confirm whether the disturbance due to shaking was the real cause of overestimation or that it was caused by increased oxygen availability. By repeating the test for different dilution factors, whether a linear type 'different dilutions vs. R' relationship would be consistently obtained can be justified. The initial DO would change in accordance with the different volumetric dilution factors, and statistical procedures can be established for QA/QC purposes to be included in the method statement for the new technique.

Note that the dilution factor may affect the outcome of the oxygen uptake rate, in the same way as it would affect the mass transfer coefficient leading to variations of the mass transfer coefficient, as shown in a typical example given by Fig. 6 in [5]. Here samples of mixed liquor feed, mixed liquor effluent, and final clarifier effluent were combined with tap water in various dilution ratios. Note that even small amounts of dirty water (e.g., 20%) caused a significant reduction in alpha α, ratio of the process water (KLaf)20 of clean diffusers to the clean water KLa of clean diffusers at equivalent conditions, which suggests either there were substantial concentrations of alpha-reducing substances that consistently depressed alpha values in the mixed liquors, or there could be other mechanisms working to reduce the mass transfer coefficient in a respiring setting.

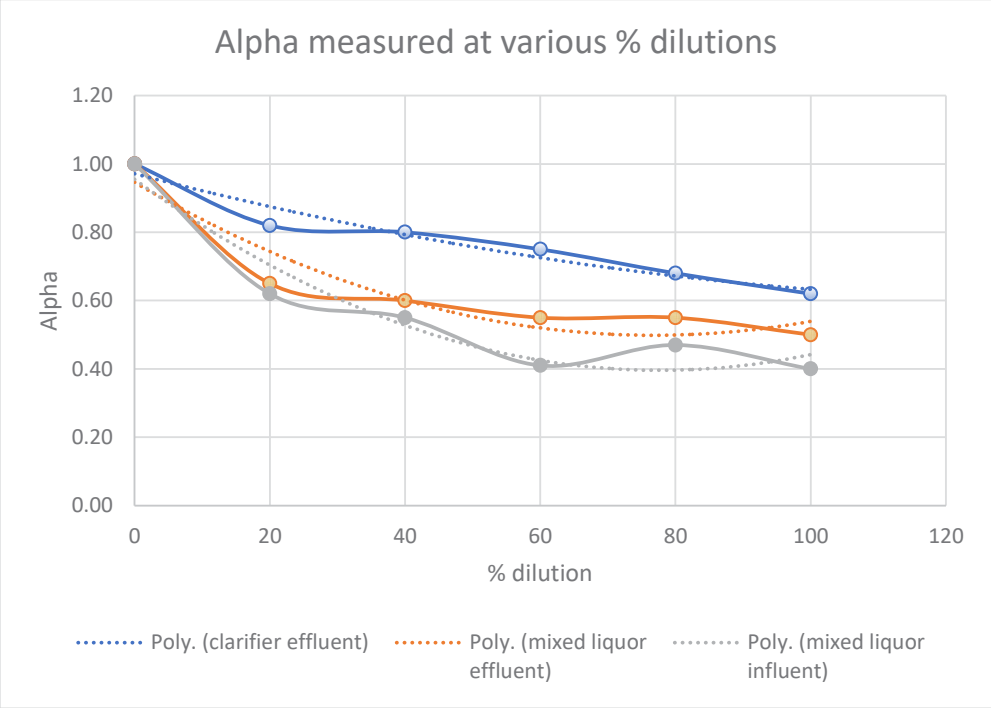

Figure 6: Dilution Test Results [Re-Constructed from Doyle et al. 1983] [5].

In other words, the resulting oxygen consumption rate may not be constant at different dilutions. However, given that there are no chemical reactions involved in the mixed liquid, trial and errors on various sample should determine which dilution mixing ratio would give the most realistic oxygen uptake rate, this method should work in any WWTP, and would avoid breaking up the floc and possibly increasing the OUR due to turbulence. There is no reason why it would 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 correct measurement of the in-situ OUR. As the graph in Fig. 1 shows, if one were to take a sample from the low DO where OUR is only 40 mv/min for example and then dilute it and provide with high DO, the OUR measured will be close to 100 mv/min (after making the dilution correction).

Effect of Erroneous Measurement of In-Process R on Calculations of the Sotrpw

The sample calculation given in ASCE (2018) can be re-constructed as below for the schematic of a completely mixed system is shown in Figure 7.

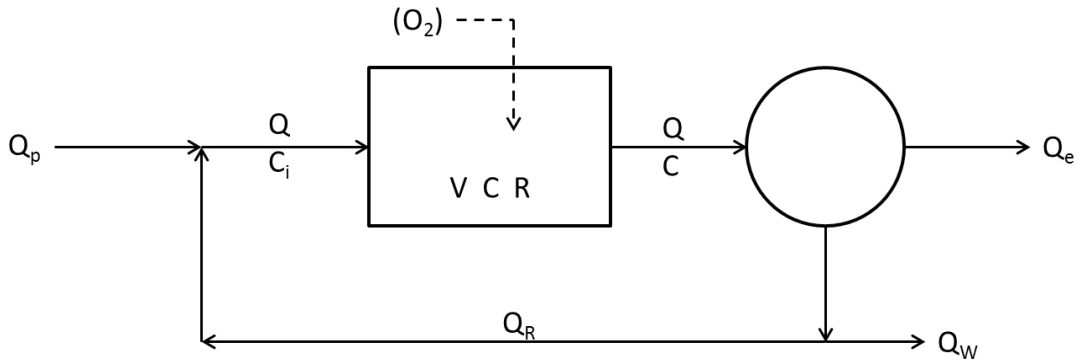

Figure 7: Schematic of a Completely Mixed System. [ASCE 2018 Figure 1-2]

The spreadsheet data in Table 2 in Appendix I (see the appendices below) pertains to Chapter 2, section 2.5, where there is a sample calculation of the oxygen transfer rate for a surface aerator with test result for Probe No. 2 during Haverstraw Test No. 8. The test was conducted in an aeration tank 3.8 m (12.5 ft) deep, and the measured average oxygen uptake rate was given as 19.4 mg/L-h. This is a complete mix basin so the OUR is 19.4 mg/L-h for the entire basin. As noted in the text, the KLa values calculated from the various probes are very uniform as would be expected in a complete mix basin. Note that this is based on averaging of multiple measurements – (see ASCE (2018) Figure 2-4). Although the method for measuring US was not discussed in detail, presumably it would be based on the BOD bottle DO depletion technique (APHA 2017 method 2710B).

Considering that the tank volume is 783 m^3, this will give an oxygen consumption rate of 19.4 X 783 X 1^{0-3} = 15.2 kg/h.

The other data collected are as follows:
Q (Total influent) = 9727 m^3/d [Qp (Primary Effluent) + QR (Return Sludge) = 6283 m^3/d +3444 m^3/d]
mixed liquor temperature = 20 ^0C
tank volume = 783 m^3
C_i (DO concentration in Q) = 2.3 mg/L
The tank detention time is calculated as:
detention time = V/Q = 783/9727 m^3/d X 24h/d = 1.93 h.

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 0C and the recorder data and calculated nonlinear regression results for Probe No. 2 is plotted out in Fig. 8 which is similarly equivalent to Fig. 2-5 in ASCE (2018) as [7]. 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 (ASCE 2022), Chapter C6, assuming a subsurface model Eq.(C6-4) gives C^*_∞=9.1 + 0.1 times diffuser submergence, in feet = 9.1 + 0.1 X 12.5 = 10.35 mg/L,
therefore, according to ASCE (2018),
the $SOTR_{pw}$ = (0.99) (10.35) (783) (3.81X10-3) = 30.5 kg O_2/h
as opposed to 26.7 kgO_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 + 0.5x (997 kg/m^3x9.81 $N/m^3/(kg/m^3)$) x 3.8m
assuming Ps = 1.013 E5 N/m2 where Ps is the surface pressure and therefore,
the mid-depth pressure gives P = 119883 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 x Y_e x P (where H is Henry's constant H = 4.3819E-4 mg/L/(N/m^2) at 20 ^0C)
C* = (4.3819 E-4) (0.19) (119883) = 9.98 say 10 mg/L
Using the modified equation that was proposed in Lee (2024), upon including the gas-depletion effect, the equation (see Appendix II) is given as:
$SOTR_{pw}$ = beta. C^*_∞. K_La_f. V- 0.001RV (1)

Therefore,
$SOTR_{pw}$ = 0.99x10x3.81E-3 x783 - 0.001x19.4 x 783 = 29.5 - 15.2 = 14.3 kg/h
the uptake rate is given by RV,
RV = 19.4x783x0.001 = 15.2 kg/h
which is not far from the transfer rate of 14.3 kg/h

This result seems to make more sense, even though we have not included the influences of the Q_R and the Q_p in the flow process. The proposed equation is therefore seeming to be valid, and the standard guideline result of SOTRpw = 26.7 kg/h is therefore inferred to be a substantial overestimation, even if R is measured correctly.

It is therefore concluded that the conventional equation without considering of the gas-phase oxygen depletion (GDP) is giving a grossly under designed system if clean water data, C^*_∞ and implicitly K_La, are used to extrapolate to field conditions without the appropriate relationships with the respiration rate.

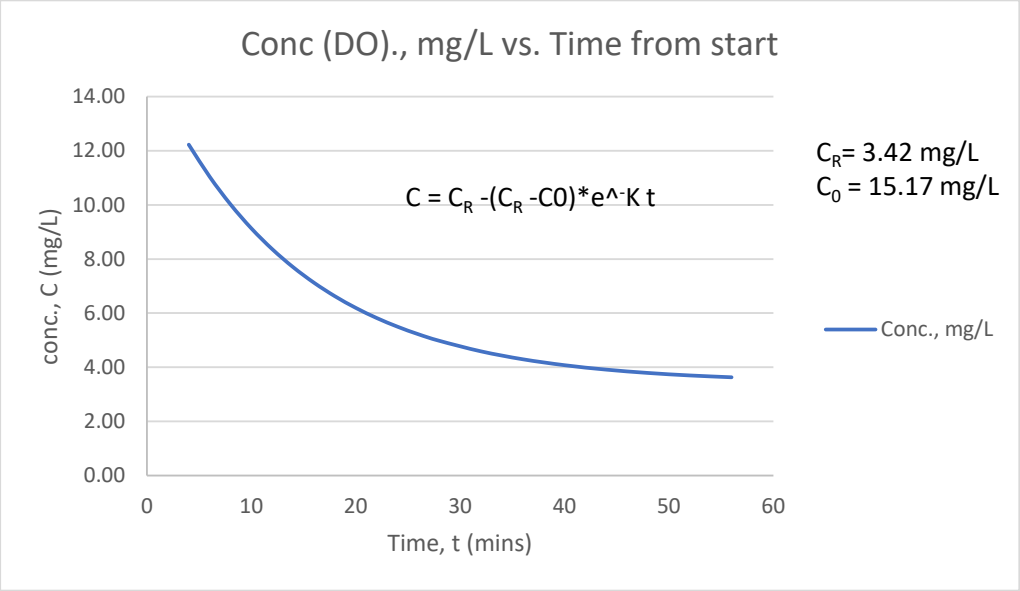

Figure 8: Nonsteady-State Oxygen Curve Based on Non-Linear Least Squares (NLLS) Fitting of data. [ASCE 2018 Figure 2-5 (Re-Constructed).]

On the other hand, it is important to recognize that the calculated "standard" SOTRpw of 30.5 kg/h is taken at 0 mg/L DO. This would be the maximum transfer rate that could be achieved when operating at 0 mg/L DO based on the calculated KLaf and using an assumed DO saturation value. This is not the actual steady state OTR that occurs when operating at a higher DO. In this case, at steady state the actual DO is 3.42 mg/L based on 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, per ASCE 2018 Eq. (2-3)
R, (mg/L/hr) = (2.3 – 3.42)/1.93 + 3.81x (0.99 x 9.09 – 3.42)
R = 20.7 mg/L/hr
where:
Cs = 9.09 mg/L (based on handbook value at 200 C)

At first it can be seen that the measured OUR of 19.4 mg/L/hr agrees reasonably well (about 6.7% different) with the calculated OUR of 20.7 mg/L/hr. It is difficult to run a steady state test during actual plant operations of course, and really there is no truly "steady state" condition. Overshadowing the problems related to the measurement of R and α, is the possibility the KLa may be dependent on uptake rate in respiring systems. If so, Eq. (2-3) may be incorrect, as it does not signify such a dependency (Lee 2024).

Practical Examples for Modification of Eq. (2-3)

An example in Garcia et al. (2010) [8]. showing the in-process model discrepancy is given in the supplemental material. The dynamic measurement of the OUR and KLa is shown in Figure S1. The calculation of CR is given in Table S1, using the Excel Solver method. Comparisons of the conventional model and the proposed model are illustrated by Eqs. (S1 to S4). This example appears to show that the OTR is related to the respiration rate R, so that Eq. (1) above applies.

Coming back to the current example, with a flow through system, OTR is not exactly OUR at steady state. One must account for the mass flow in and out of oxygen, which Eq. (2-3) does. Actually, as Eq. (2-3) states:
R = (Ci – C)/HRT + OTR (2)

where OTR is KLaf (Cs – CR), and only when Ci = C does OTR = R. However, the term (Ci – C)/HRT is small compared to KLaf x (Cs – CR), giving 0.58 vs. 26.00, for the two terms.
The calculated OUR that was shown does include the (mass of oxygen) in less massout [the term (Ci – CR)/HRT]. And the calculated value for R using the non-steady state analysis agrees with the measured value of R very well.

If one wants to consider the mass basis, the mass rate of oxygen consumption is:
Based on measured R value, OUR = 19.4 mg/L/hr x 783 m3 /1000 = 15.2 kg/hr
Based on Eq. (2-3), OUR = 20.7 mg/L/hr x 783 m3 / 1000 = 16.2 kg/hr
The above calculation seems to have justified Eq. (2-3) and so it would seem that gas-side depletion (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) so happened to give the same R as the R measured (by BOD bottle) is a coincidence, aggravated by the assumed saturation concentration, taken as 9.09 mg/L which is unjustified.

With the proposed modifications [Lee 2022, 2024] Eq. (2-3) becomes
$2R = (Ci-CR)/HRT + KLaf (C^*\infty f - CR)$ (3)
i.e.,
$2R = (2.3 -3.42)/1.93 + 3.81(10.35 X 0.99 -3.42)$
[Note that $C^*\infty f$ is not 9.09 mg/L but 10.35 mg/L]
giving,
-0.58 + 26.00 = 2R, therefore,
R = 25.4/2 = 12.7 mg/L-h

This shows that the BOD bottle method gave too high an R and the value of 19.4 mg/L-h is not correct if the modified equation is correct. The true SOTRpw at C = 0 mg/L should be somewhat like, assuming R has not changed, 12.7 x 783 x 10-3 = 9.95 kg/h << 30.5 as calculated previously showing the great anomaly 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-h) as much as almost twice its true value. This is very unfortunate, as the ASCE-calculated value of 26.7 kg/h will never match up with any calculated R consumption rate and the discrepancy will only increase the more high-rate the treatment is (i.e., higher OUR). The point being the higher the biochemical reactions undergoing in the basin, with the same gas flowrate, the less will be the SOTRpw because of the gas depletion effect, and this can be verified by many tests already done by many investigators, such as Lopez et al. (2006), Garcia-Ochoa et al, (2010) and so forth [8].

Of course, SOTRpw – 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 X 9.09 – 3.42 = 5.58 mg/L. The calculation of SOTRpw is not the actual oxygen transfer rate at steady state. The calculation at 0 mg/L uses a driving force of 0.99 X 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. So, if the ASCE equation [Eq. (2-3)] is deemed correct giving an OUR of 16.2, then 16.2 x 9/5.58 = 26.1 kg/hr – at 0 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 old method with the modified, we have as shown in Table 1, where the true driving force (DF) at steady state is (C* - CR) but C* is given by:
$C^* = C_{(\infty f)}^* - R/KLaf$ (4)
Therefore, C* = 10.0 X 0.99 – 12.7/3.79 = 6.55 mg/L
Therefore, DF (SS) = 6.55 - 3.42 = 3.13 mg/L

	conventional method	modified Eq. (2-3)
OUR (APHA)	19.4/20.7 mg/L-h	-
OUR (dilution)	-	12.7 mg/L-h
SOTRpw (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
SOTRpw (calc.)	1.6X16.2=26 kg/h	3.16 X 9.9 = 31.3 kg/h

Table 1: Comparison of the two Models

The discrepancy in the new method between the measured and the calculated SOTRpw 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 mg/L-h which is incorrect because of 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 mg/L-h instead of 19.4 mg/L-h; and secondly the incorrect driving force at the steady state, making the OTR at test conditions erroneously high. Although the calculated value using Eq. (2-3) seems to match the measured value, both of which are incorrect, the end result would be a KLa value being estimated low, if the old equation using the measured R is used. This is evident from ASCE (2018) Table 5-2 that compares the KLaf values between the SS (steady state) and the NSS (non-steady state) methods for various on-site measurements.

The controversial issue is that Cs*∞f is a lot higher than 9.09 mg/L in a surface aeration system. When clean water tests are conducted with Orbal disc aerators (Doyle 2024) the clean water saturation is very close to the surface saturation at that temperature, and any significant increase in Cs due to subsurface entrainment is not observed with that particular aeration device. In a diffused air system for sure one will see the increase due to depth of the air release of course. Perhaps also in some other surface aeration systems which inject bubbles below the surface there is an entrainment effect. In testing in respiring systems over many years, although it has been found that the standard ASCE equation matches up with data quite well for the Orbal aeration system and no consistent findings in literature to suggest that there should be a 2R term in the mass balance equation in a respiring system have ever been observed, the actual error could be a combination of the various effects, paramount of which is the gas-side gas depletion. To fully convince the scientific community, it may be necessary to show that gas phase gas depletion (GDP) exists (or not) via carefully controlled tests.

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. (2005), the mass transfer coefficient of biological floc (KLabf) was estimated from the mass transfer coefficient of the mixed-liquor (KLaf) and the reactor-solution (KLae). The biological floc resistance (BFR) and reactor-solution resistance (SR) were defined as the reciprocal of KLabf and KLae, respectively, by applying the concept of serial-resistance originally presented in two-film theory (Lewis and Whitman 1924). The specific biological floc resistance (SBFR) was defined as biological floc resistance per unit biomass concentration. The data indicated that an activated sludge process yielding low BFR/MLR and BFR/SR ratios tended 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 run. Furthermore, SBFR successfully represented biological floc and showed a positive correlation to sludge volume index (SVI). In addition, the ratio SBFR/SR and oxygen transfer efficiency (OTEf) 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 (2022). The results of this re-investigation showed that the oxygen transfer rate is affected by the microbial respiration rate that would result in a modification of the ASCE (2018) Eq. (2-3) giving a 2R term instead of a single R term.

Possibility of Over-Estimation

Hines et al (1975) 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/m3 aeration - tank volume.hr [9,10]. 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 O2/m3-hr, assuming that the concentration of mixed liquor suspended solids was 5000 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/m3-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. "If you reduce a dissolved oxygen set point from 2.5 mg/L to 2.2 mg/L, you can actually generate up to 20% energy savings," said Michael Dooley, CEO at instrument provider and consultancy Strathkelvin Instruments. "By being able to only supply the oxygen that you need to a DO level you need and to keep that at a minimum, you have a much more efficient process and the bacteria are much happier. It doesn't sound like a lot, but it is." 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 insitu oxygen uptake rate for the microbial respiration, and so it is important not to disturb the actual DO concentration in a sample prior to measurement to avoid the possibility of over-estimation.

Discussion and Conclusion

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, measurement of the 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 (ASCE 2018). This paper presents an argument that the inaccurate measurement maybe due to the agitation of the sample liquid that shears the biofloc that exposes the microbes to higher respiration rates, and offers a solution. As well, the microbial stress due to the agitation may in turn increase the respiration rate which is undesirable [11-19].

To extricate the experiment from this predicament, it is recommended that the sample would be subject to pure water dilution (or tap water dilution) to produce a high DO content without the agitation, in contrast to the conventional method that is stipulated in APHA 2017. Furthermore, with the modified equation for oxygen transfer replacing Eq. (2-3) in ASCE (2018) [Lee 2024], it is expected that a more accurate measurement as described in the dilution method should match the calculated result by the modified equation for the actual oxygen requirement (AOR) from which the standard oxygen requirement (SOR) can be calculated.

Once we have an SOR, an 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 (200 C, 1 atm, 0 mg/L DO). In this case 26.7 kg/hr is not truly a "standard" condition because the KLa was determined in dirty water in the presence of microbial respiration, and is not a clean water mass transfer coefficient, although the DO is 0 and temperature is 20 0C which is indeed already at standard conditions. There would have to be a design alpha value (α) applied to calculate a clean water KLa. With surface aeration that alpha factor is usually close to 1 for domestic sewage that we usually take as about 0.9-0.95. According to Doyle (2024) 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 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 appendix and the subsequent calculations, and a diffused aerator as shown in the supplemental material appear to validate both Eq. (1) and Eq. (4). Therefore, it may be advisable to repeat this ASCE experiment, and to confirm that the true respiration rate, R, would be 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 3. Furthermore, the SOTRpw of 30.5 kg/h would replace 26.7 kg/h as given in the ASCE (2018) estimation at zero DO and for when a steady-state of R = 19.4 mg/L-h (this value widely suspected to be incorrect) is attainable; but the true SOTRpw should be given by 12.7 x 783 x 10-3 = 9.95 kg/h at 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 at test conditions only, the SOTRpw would be calculated to be 31.3 kg/h and not 26.7 kg/h, when R = 0 when the DO = 0. A lot depends on whether SOTRpw 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 for the contents in Table 1.

According to ASCE (2018), additional discrepancies between in-situ R and ex-situ estimates happen when the ex-situ R 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 a DO of 0.5 and 2.0 mg/L. Using the previously developed biological model [not stated], 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 resulted in a 50% increase in nitrogenous OUR in the ex-situ device.

To alleviate the many problems of measurement for 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 2017. With a more accurate measurement of the OUR, it may lend credence to the modification of the fundamental equation for oxygen transfer [Lee 2024] in a respiring system, as applied to an example provided by ASCE/EWRI 18-18 recently published.

Appendices

Appendix I

K(1/h)		Summary Data							
4.33534	K, C_R, C_0		Time, (min)	Conc., mg/L	Fit Value	Residual	conc. (modeled)	delta	(delta)^2
K (min-1)	0.072256	1	4	12.176	12.2247	-0.0486956	12.22	-0.05	0.002
CR (mg/L)	3.42313	2	6	11.008	11.0404	-0.0324011	11.04	-0.03	0.001
C0(mg/L)	15.17429	3	8	10.144	10.0155	0.12854	10.02	0.13	0.017
		4	10	9.104	9.12843	-0.0244293	9.13	-0.02	0.001

		5	12	8.32	8.36076	-0.0407534	8.36	-0.04	0.002
		6	14	7.792	7.69637	0.0956278	7.70	0.10	0.009
		7	16	7.072	7.12139	-0.04386	7.12	-0.05	0.002
		8	18	6.624	6.62377	2.32E-04	6.62	0.00	0.000
		9	20	6.24	6.19311	0.0468936	6.19	0.05	0.002
		10	22	5.76	5.82039	-0.0603924	5.82	-0.06	0.004
		11	24	5.52	5.49783	0.02217515	5.50	0.02	0.000
		12	26	5.248	5.21867	0.0293322	5.22	0.03	0.001
		13	28	4.96	4.97707	-0.0170693	4.98	-0.02	0.000
		14	32	4.56	4.58702	-0.0270233	4.59	-0.03	0.001
		15	36	4.256	4.29488	-0.0388803	4.29	-0.04	0.002
		16	40	3.952	4.07607	-0.124066	4.08	-0.12	0.015
		17	44	3.872	3.91218	-0.0401764	3.91	-0.04	0.002
		18	48	3.84	3.78942	0.0505767	3.79	0.05	0.003
		19	52	3.776	3.69748	0.0785182	3.70	0.08	0.006
		20	56	3.68	3.62862	0.0513816	3.63	0.05	0.003
									0.072

Table 2: Nonlinear Estimation for Nonsteady-State Oxygen Transfer: Haverstraw Run No. 8, Probe No. 2 Data
(Note: The Recorder data and Calculated Nonlinear Regression Results for Probe No. 2 is Plotted out in Fig. 8 which is Similar to Fig. 2-5 in ASCE 2018.)

Appendix II
Modified version of Eq. (2-3) used for the nonsteady-state method (now Eqs. (1 and 4))
What was proposed in Lee (2024) is that C* should be a function of R, or
$C^{*} = C^{*}_{\infty} - R/K_L a_f$
For the transfer equation as given by Magdalena et al. (2022): $dC/dt = KLa (C^* - C) – R$,
substituting the value of C* into the transfer equation gives
$dC/dt = KLa ((C^*_{\infty} f - R/K_L a_f) - C) - R$
when at steady state, $dC/dt = 0$, and $C = CR$, $KLa = K_L a_f$ so that
$K_L a_f = R/(C^*_{\infty f} - R/K_L a_f - C_R)$
expanding the equation gives
$R = K_L a_f . C^*\infty_f - R - K_L a_f C_R$
Therefore,
$2R = K_L a_f (C^*_{\infty_f} - C_R)$
as opposed to ASCE (2018) Eq (2-3)
for a batch process which only gives $R = K_L a_f (C^*_{\infty_f} - C_R)$

It is important to mention that the KLa coefficient is an empirical value that must be estimated or experimentally assayed (typically in an abiotic setting). KLa 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 KLa.

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

Conflict of Interest Statement
Authors are 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.

Supplemental Material
Supplemental Material text, Figs. S1; Table S1; equations S1-S4 are available online.
The authors declare no conflict of interest.

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Supplemental material

It would appear that between the old model and the newly proposed model, wherein

$dC/dt = KLa1 (C^* - C) – OUR$; conventional

$dC/dt = KLa2 (C^* - C) – 2\times OUR$; Lee

that, in which C* is considered constant, to reconcile these two equations, KLa1 = KLa2, but that does not seem to be possible if both models are considered correct. In reality the conventional model ignores any effect of gas-side oxygen depletion which is reasonable only if gas supply >> OUR. However, it is correct in saying that KLa1 (in the old model) and KLa2 (in the new model) are identical in a non-steady state (NSS) test, since the form and structure of the equations are the same in both formulae, but the discrepancy is in CR if R is measured, or in R if CR is measured. Garcia et al. [2009] compared two determination methods for the oxygen uptake rate R, namely the dynamic method and the oxygen profile data method for a fermentation broth. In terms of estimating the oxygen uptake rate (OUR) and KLa, the methods are similar to the steady-state method (in the measurement of OUR), and the non-steady state method (in the measurement of KLa) respectively, as given by ASCE/EWRI 18-18. In the dynamic method example, as described by Garcia and as shown in Fig. S1, the airflow inlet to the fermentation broth is interrupted for a few minutes so that a decrease of DO concentration can be observed. 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 slope from after the stopping of the air flow, and the procedure repeated several times for precision. In their tests, the R is measured insitu and both R and CR 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. 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 KLa 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 KLa. Contrasting with the profile data method where the KLa 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). 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 be the same as the dynamic method in this example. But 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.

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 = KLa (C* - C) – OUR (S1)
C = CR - (CR - C0) exp (-KLa.t) (S2)

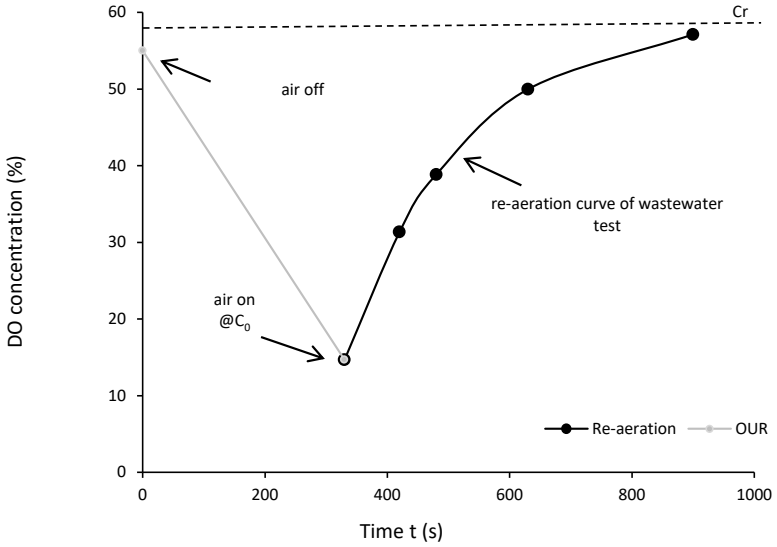

Fig. S1. Dynamic measurement of OUR and KLa

From Fig. S1, it can be read the following:
CR = 59.5%; C0 = 14.7%, therefore, substituting the readings into eq S2, we have
C ≈ 59.5 - (59.5 - 14.7) exp (-0. 0052.t)
where KLa is found to be 0.0052 s-1 by fitting the read data to the model by the non-linear least square (NLLS) method, using the Excel solver as shown in the calculation table (Table S1) below. Garcia's own calculation in their report gave: KLa = 0.0057 s-1. Using Eq. S1, if the steady state concentration (C = CR) is taken to be around 55%, then

R would be calculated as
R = 0.0057. (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 (S3)
Therefore, R ≈ (55-13)/335*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 KLa obtained is only one-half of that obtained by the non-linear least squares (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 effect, Equation (S1) should be replaced by previously stated Eq. (43):
dC/dt = K1 (K2 – C) - r – R
when the resistance r is equated to R, K1 replaced by KLaf and K2 replaced by C*∞f

The equation becomes similar to Eq. (44) as
dC/dt = KLaf (C*∞f - C) -2R (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:
KLaf = 2 x 0.125/ (100 – 55) = 0.0056 s -1
which is exactly the same as measured by the conventional equation used by Garcia et al. for the profile method (same as the NSS method), that would give R = 0.256% s-1 which gives twice the value measured in-situ by the linear decay curve. Garcia et al. assumed that the decay-curve measured R value is deemed invalid (cell economy principle) due to an alleged lowering of the consumption rate [Garcia-Ochoa et al. 2009]. However, there does not seem to have a foundation for such a cell economy principle unless oxygen-limiting conditions exist or that the cells are in an anaerobic

state--- neither of these conditions would have happened in this test. [ASCE 2018]

start time		time from start (s)		duration (s)	c (%)	Cr, KLaf, C0	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		

Table S1. Re-constructed data from Garcia et al

Garcia's equation is identical to the ASCE equation given as: (Note that C* in his equation is used in the context of C*∞) Eq. (S1) and Eq. (S2).
In a non-respiring system, C* must be close to CR. The superposition principle, also known as superposition property, states that, for all linear systems, the net response caused by two or more stimuli is the sum of the responses that would have been caused by each stimulus individually. The above equation is only correct if OUR does not affect the transfer rate.

However, since the OUR does impact on the transfer rate or the dissolution rate by as much as R, the oxygen transfer rate is a superposition of two mass motions. The net transfer can be described as a combination of two separate motions: the two-film transfer motion based on the two-film theory without microbial respiration, and microbial cell oxygen demand inducing an oxygen transport without the film transfer. This makes dC/dt = dW/dt - R in a respiring system, as opposed to dW/dt = dC/dt in a non-respiring one. Since this principle is always true scientifically, it does not matter whether the aeration system is surface or subsurface. The resulting equation dC/dt = KLa (C* - C) – 2 X OUR must always be true if the parameters are interpreted as measured in a non-respiring system