A Restructured Framework for Modeling Oxygen Transfer in Two-Phase Partitioning Bioreactors

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Abstract: This communication proposes a mechanistic modification to a recently published method for analyzing oxygen mass transfer in two-phase partitioning bioreactors (Nielsen et al., 2003), and corrects an oversight in that paper. The newly proposed modification replaces the earlier empirical approach, which treated the two liquid phases as a single, homogeneous liquid phase, with a two-phase mass transfer model of greater fundamental rigor. Additionally, newly developed empirical models are presented that predict the mass transfer coefficient of oxygen absorption in both aqueous medium and an organic phase (n-hexadecane) as a function of bioreactor operating conditions. Experimental values and theoretical predictions of mass transfer coefficients in two-phase dispersions, $k_L a_{TP}$, are compared. The revised approach more clearly demonstrates the potential for oxygen mass transfer enhancement by organic phase addition, one of the motivations for employing a distinct second phase in a partitioning bioreactor. © 2005 Wiley Periodicals, Inc.

Keywords: oxygen transfer; $k_L a$; two-phase partitioning bioreactor

INTRODUCTION

The addition of organic solvents to bioreactor systems has been shown to improve oxygen transfer by both enhancing the mass transfer coefficient (Hassan and Robinson, 1977), as well as the equilibrium driving force (Ho et al., 1990). The average driving force in a two-phase system is increased by adding a solvent with greater dissolved oxygen solubility than the aqueous medium. One such popular organic phase, n-hexadecane, has a dissolved oxygen solubility reported to be between 8 and 11 times that of water at $30°C$ (Ju and Ho, 1989).

Oxygen absorption in a two-phase partitioning bioreactor containing n-hexadecane has been previously studied by the authors (Nielsen et al., 2003). This study resulted in the development of an empirical model for predicting oxygen absorption mass transfer coefficients as a function of the traditional operating parameters of power input and aeration

rate, but also as a function of the organic phase volume fraction. The original model assumed that the liquid–liquid two-phase system could be represented as a single homogeneous liquid phase with uniform properties. This simplification depends upon the assumption that the partitioning mass transfer of dissolved oxygen between the two liquid phases was rapid enough to consider the system to be at equilibrium. Suitability of this assumption has been successfully demonstrated in numerous studies while working with aqueous—n-hexadecane two-phase systems (Ho et al., 1990; Linek and Benes, 1976; McMillan and Wang, 1990). Furthermore, Zhao et al. (1999) found the rate of dissolved oxygen partitioning mass transfer between the two liquid phases of the aqueous—n-hexadecane system to be, "on average, three times larger" than the rate of oxygen absorption from the gas phase, suggesting that the latter contributes much more to the overall mass transfer resistance. In the authors' previous study, this assumption led to the introduction of a weighting factor, w, to account for the difference in oxygen solubility in the two-phase mixture as a result of organic addition:

$$\frac{dC_A}{dt} = k_L a \cdot w \cdot (C_A^* - C_A) \tag{1}$$

In Equation 1, $k_L a$ represents the bulk, two-phase mass transfer coefficient measured in the well-mixed, liquid–liquid dispersion and was directly measured as a function of aeration and agitation rates, as well as the organic phase volume fraction, X_{ORG}. The weighting factor is a function of the organic volume fraction selected, and was defined as follows:

$$w = \frac{V_A + D_{OA} \cdot V_O}{V_A + V_O} = (1 - X_{ORG}) + D_{OA} \cdot X_{ORG} \tag{2}$$

where D_{OA} is the dissolved oxygen partitioning coefficient between the organic and aqueous phases. Note that w takes on

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a value ranging from 1 to D_{OA} as the organic volume fraction, X_{ORG}, varies between 0 (single aqueous phase) and 1 (single organic phase). A closer examination, however, identifies a fundamental flaw in the previously proposed model. Equation 1 does not correctly weight the accumulation term on the left-hand side of the equation to account for the difference in the oxygen solubility within the two-phase dispersion, as was done on the right-hand side of the equation. Therefore, to consistently account for the effect of the organic phase on oxygen solubility in the dispersion throughout the model, Equation 1 should be written as:

$$ w \cdot \frac{dC_A}{dt} = k_L a \cdot w \cdot (C_A^* - C_A) \qquad (3) $$

which can be further simplified to eliminate the appearance of w. Equation 3 now predicts that, at any constant set of operating conditions in the two-phase system, the mass transfer coefficients between the gas and each of the aqueous and organic phases will be equivalent to the effective mass transfer coefficient measured for the liquid–liquid dispersion. This result is a direct consequence of the assumption of instantaneous dissolved oxygen partitioning between the liquid phases made when developing the model. Note that Equation 3 still predicts that the total rate of oxygen absorption into the two-phase system will be greater in the presence of an organic phase with a higher affinity for dissolved oxygen. The effect of the organic phase on the total rate of oxygen transfer in the two-phase system is now explicitly described in Equation 3, accounting for influences in both the driving force and the mass transfer coefficient when combined with the empirical relationship for $k_L a$ of Nielsen et al. (2003).

To improve the fundamental nature of the original model, we now propose the use of a heterogeneous mass transfer model to describe oxygen absorption in a two-phase partitioning bioreactor. The model, shown in Equation 4, still relies upon the assumption that the two liquid phases are at equilibrium.

$$ (V_A + D_{OA} \cdot V_O) \cdot \frac{dC_A}{dt} = k_L a_A \cdot (C_A^* - C_A) \cdot V_A $$
$$ + k_L a_O \cdot D_{OA} \cdot (C_A^* - C_A) \cdot V_O \quad (4) $$

Equation 4 is expected to remain valid provided that well-mixed conditions are maintained in the two-phase system, such that the rates of partitioning mass transfer between the liquid phases can be rapid enough to warrant an equilibrium assumption. Obtaining unique parameter estimates for the individual lumped volumetric mass transfer coefficients, $k_L a_A$ and $k_L a_O$, is difficult in a two-phase system as these parameters are not identifiable at a constant organic fraction, X_{ORG}. This fact is clearly illustrated by collecting the constant terms of Equation 4:

$$ (V_A + D_{OA} \cdot V_O) \cdot \frac{dC_A}{dt} = (k_L a_A \cdot V_A + k_L a_O \cdot D_{OA} \cdot V_O) $$
$$ \cdot (C_A^* - C_A) \qquad (5) $$

which could be rearranged and expressed as:

$$ \frac{dC_A}{dt} = k_L a_{TP} \cdot (C_A^* - C_A) \qquad (6) $$

where:

$$ k_L a_{TP} = \frac{(k_L a_A \cdot V_A + k_L a_O \cdot D_{OA} \cdot V_O)}{(V_A + D_{OA} \cdot V_O)} \qquad (7) $$

Difficulties estimating individual parameters in the two-phase system are due to the fact that equivalent predictions of the effective two-phase mass transfer coefficient can be attained for different combinations of $k_L a_A$ and $k_L a_O$ at a given constant organic volume fraction. This problem is most easily overcome by isolating $k_L a_A$ and $k_L a_O$ in single phase experiments. Note that $k_L a_{TP}$, the effective mass transfer coefficient of the dispersed two-phase system (analogous to $k_L a$ in Eq. 3), is a function of X_{ORG} at any constant combination of agitation and aeration rates. However, $k_L a_{TP}$ is now fundamentally derived by explicitly modeling the constituent mass transfer phenomena in the two-phase system, where previously an empirical approach had been used. Furthermore, although Equation 6 has been simplified to an expression of the same structure as Equation 3, the simplifications made earlier in Nielsen et al. (2003) in order to model the system as a single, homogeneous phase are no longer required.

MATERIALS AND METHODS

New experiments were conducted using a procedure identical to that previously performed by the authors (Nielsen et al., 2003), however, now with a greater total liquid volume of 3 L in the larger 5 L New Brunswick BioFlo III bioreactor ($D_T = 17$ cm). Again, two six-blade Rushton turbine impellers ($D_I = 7.7$ cm, $H_I = 1.5$ cm, 5 cm spacing) were used together with four baffles to enhance mixing. Air was supplied beneath the impellers via a sparger. The aqueous medium and organic phase, n-hexadecane, were obtained and prepared as described in the authors' previous work. All analytical equipment and sampling procedures are identical between the two studies.

The agitation and aerations rates used in the present study vary slightly in value from those of the past study, as they represent combinations that were better suited for use at the larger bioreactor scale and with the current agitation equipment. Aeration of the bioreactor was conducted in 3 L of both aqueous medium and n-hexadecane alone, and also on two-phase systems containing 10%, 20%, and 33% (by volume) organic phase for comparison with theoretical predictions. Agitation rates of 400, 600, and 800 rpm were systematically combined with all combinations of aeration rates that varied between 1, 2, and 3 L/min for each of the liquid phase compositions.

Estimates of the individual mass transfer coefficients at each set of conditions were obtained by nonlinear least-squares regression using the Gauss–Newton method with Matlab$^\circledR$ on a second-order absorption model that accounted

for probe response dynamics. Estimates of power requirements at each set of operating conditions were obtained by following the methods outlined in Nielsen and Villadsen (1994). The intrinsic Matlab® function NLINFIT was again used to satisfy the least-squares criteria using the Gauss–Newton method in order to estimate the parameters of each of the nonlinear $k_L a$ models.

RESULTS AND DISCUSSION

Single-Phase Absorption

Data collected in Tables I and II were used to fit empirical models of the following familiar form (Nielsen and Villadsen, 1994) for both the aqueous and organic phases:

$$k_L a_i = \alpha \cdot \left(\frac{P_g}{V}\right)^{\beta} \cdot (v_s)^{\gamma} \qquad (8)$$

where δ, α, and β represent empirical parameters to be estimated, P_g represents the power input to the aerated bioreactor in W, V represents the total liquid working volume in the bioreactor in m³, v_s represents the superficial gas velocity through the bioreactor in m/s, and i is equivalent to either A or O for aqueous and organic experiments, respectively.

Parameter estimates were obtained for each data set after transforming the independent variables into the more commonly used forms of predicted power inputs, P_g/V, and superficial gas velocities, v_s. Parameter estimates of α, β, and γ were obtained as 284, 0.21, and 0.49 for the aqueous phase and as 15570, −0.07, and 0.71, for the organic phase. It is clear from the data shown in Tables I and II, as well as from the parameter estimates obtained, that the two phases show notably different responses to the operating conditions, in particular to changes in agitation rate. Nevertheless, it is clear from Figure 1 that for both phases, the model combined with each respective set of parameters is able to predict well the mass transfer coefficient over the range of operating conditions investigated. Residual analyses (not shown) suggest that no significant functionality was neglected in either case.

The agitation equipment and configurations associated with the current bioreactor arrangement allow for much greater power transfer to the liquid phases, resulting in estimates of P_g/V that were nearly ten times that of the authors' earlier study at all comparable settings. Greater power dissipation into the liquid phases leads to greater mixing in the bioreactor, corresponding to improved dispersions of both liquid and gas phases. Not surprisingly,

Table I. Experimental aqueous phase $k_L a_A$ (/h) data.

Agitation rate (rpm)	Aeration rate (L/min)		
	1	2	3
400	59.2	76.4	84.9
600	74.8	122.9	129.4
800	78.4	127	143.4

Table II. Experimental organic phase $k_L a_O$ (/h) data.

Agitation rate (rpm)	Aeration rate (L/min)		
	1	2	3
400	50.8	92.1	121.3
600	40.9	76.8	110.5
800	36.5	71.9	101.9

aqueous phase $k_L a_A$ values measured at identical combinations of aeration and agitation rates were significantly higher in the present study. Although greater total volumetric aeration rates were examined in the present study, lower superficial gas velocities resulted. The volumetric aeration rates used in the present study do, however, correspond to anticipated conditions that would arise during actual operation of a two-phase partitioning bioreactor.

Two-Phase Absorption

Experimental values of $k_L a_{TP}$ are compared with theoretical values calculated using the data of Tables I and II, a D_{OA} estimate of 7.53 mg/L organic/mg/L aqueous (Hassan and Robinson, 1977), and Equation 7. The results are shown in Figure 2a–c for organic volume fractions of 10%, 20%, and 33%, respectively.

From Figure 2a–c it appears that the experimental results confirm the suitability of Equation 7 over the range of conditions studied, apart from a few obvious exceptions. The agreement between the equilibrium model and the experimental data begins to depart as the aeration rate is increased and the model over-predicts the corresponding $k_L a_{TP}$ values. This behavior is amplified as the organic fraction is increased. For instance, as the organic fraction is increased from 10% to 20%, and finally to 33% at 3 L/min and 800 rpm, predicted $k_L a_{TP}$ values vary from their experimental counterparts by 7%, 18%, and 28%, respectively. As the organic fraction was increased, foaming became a major issue.
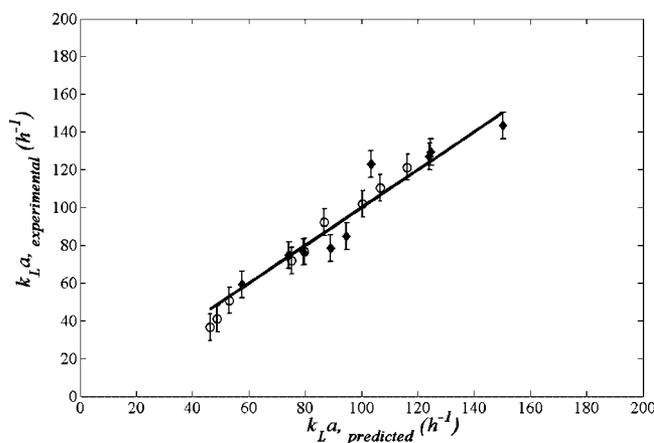
Figure 1. Experimentally obtained, and model predicted, $k_L a_A$ (◆) and $k_L a_O$ (○) values over a range of operating conditions compared to a line of slope 1. Error bars shown at one standard deviation (±7/h).
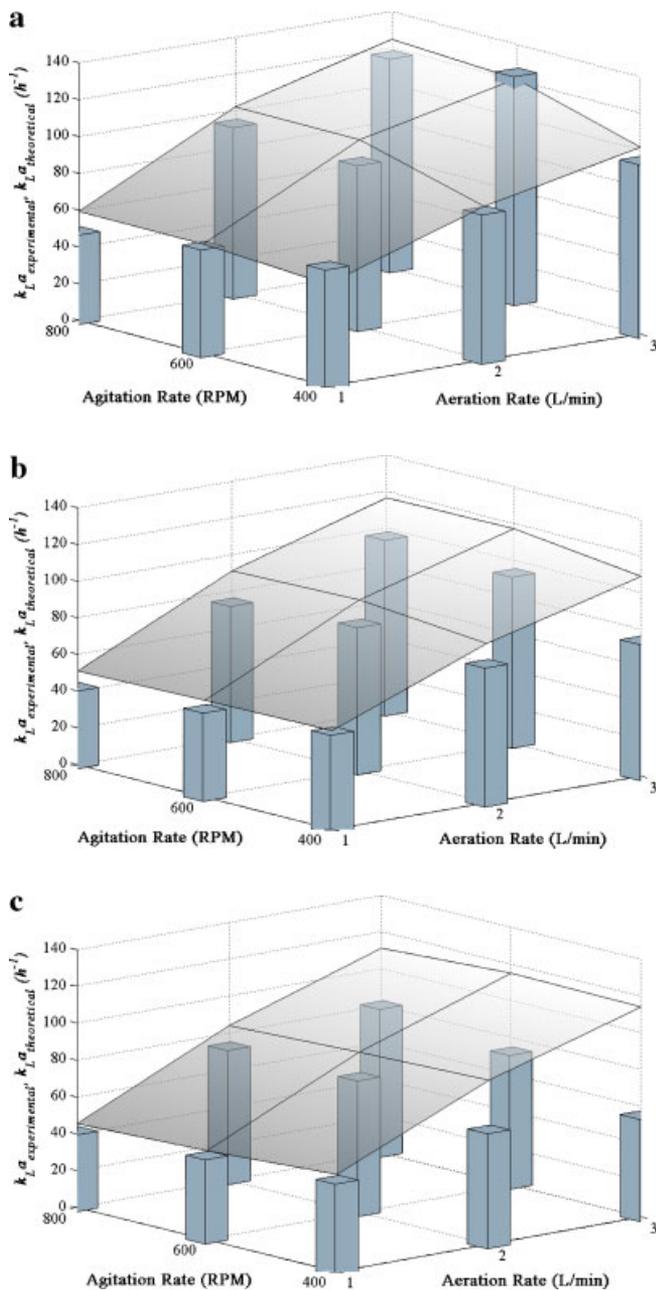
Figure 2. **a**: Comparison of experimental (bars) and theoretical (surface) effective two-phase oxygen mass transfer coefficients, k_La_{TP}, at various agitation and aeration rates and 10% organic phase (by volume). **b**: Comparison of experimental (bars) and theoretical (surface) effective two-phase oxygen mass transfer coefficients, k_La_{TP}, at various agitation and aeration rates and 20% organic phase (by volume). **c**: Comparison of experimental (bars) and theoretical (surface) effective two-phase oxygen mass transfer coefficients, k_La_{TP}, at various agitation and aeration rates and 33% organic phase (by volume). [Color figure can be seen in the online version of this article, available at www.interscience.wiley.com.]

During the most aggressively aerated experiments the foaming was so great that reactor contents were even forced out the top of the vessel. Excessive foaming, resulting in greater gas entrainment and a lower liquid volume, could affect energy distribution in the bioreactor. These settings obviously represent unsuitable conditions from an operating perspec-

tive, and they account for poor agreement between the model predictions and experimental data. The experimental results validate the assumption of two-phase partitioning equilibrium of dissolved oxygen in this system, particularly under less aggressive aeration conditions, such as those employed in normal operation of this system.

Given the relatively wide disparity between literature estimates of D_{OA} for dissolved oxygen between *n*-hexadecane and water, varying between 6 (McMillan and Wang, 1990) and 11 mg/L organic/mg/L aqueous (Ju and Ho, 1989), it is possible that the agreement between experimental and theoretical predictions of k_La_{TP} could be improved with a more accurate estimate of D_{OA}. As seen in Equation 7, D_{OA} determines how the relative effect of the mass transfer coefficient of each individual liquid phase influences the overall dispersion. Sensitivity of k_La_{TP} predictions to D_{OA} estimates will be the subject of future studies to determine the value of an improved estimate.

Effect of Organic Phase

The total rate of oxygen absorption in the two-phase system, $\dot{m}_{TP}$, is equivalent to the balance given by Equation 4, while the total rate of oxygen transfer in a bioreactor containing a single aqueous phase of equal liquid working volume, $\dot{m}_A$, is given by:

$$\dot{m}_A = k_La_A \cdot (C_A^* - C_A) \cdot V_T \qquad (9)$$

As such, the ratio of the total rates of mass transfer between the two-phase and single aqueous phase systems can be calculated as:

$$\frac{\dot{m}_{TP}}{\dot{m}_A} =$$

$$\frac{k_La_A \cdot (C_A^* - C_A) \cdot V_A + k_La_O \cdot D_{OA} \cdot (C_A^* - C_A) \cdot V_O}{k_La_A \cdot (C_A^* - C_A) \cdot V_T}$$

$$(10)$$

where V_T is the total volume of the aqueous-organic dispersion, equivalent to the sum of V_A and V_O in the two-phase system. Simplifying and substituting for the definition of X_{ORG} yields Equation 11:

$$\frac{\dot{m}_{TP}}{\dot{m}_A} = 1 + \left(\frac{k_La_O \cdot D_{OA}}{k_La_A} - 1 \right) \cdot X_{ORG} \qquad (11)$$

From Equation 11 it is clear that the ratio $\frac{\dot{m}_{TP}}{\dot{m}_A}$ will be greater than 1, implying that an enhanced total rate of transfer to the bioreactor will be achieved in the presence of a second phase, provided the following condition is satisfied:

$$k_La_O \cdot D_{OA} > k_La_A \qquad (12)$$

From the experimental values found for k_La_A and k_La_O in Tables I and II, respectively, the mass transfer coefficients of the individual phases were never observed to differ from each other by much more than a factor of 2 at any set of operating conditions examined. Therefore, considering a typical value of D_{OA} of 7.53 mg/L organic/mg/L aqueous

(Hassan and Robinson, 1977), Equation 12 will be satisfied over the entire range of operating conditions studied here. It can be predicted from these results that the addition of any quantity of organic phase with an enhanced affinity for dissolved oxygen will promote a higher total rate of oxygen transfer to the liquid phases of a partitioning bioreactor. Furthermore, from Equation 11 it is clear that the enhancement will be directly proportional to the organic volume added, a conclusion that is consistent with findings of McMillan and Wang (1990). Enhancing the total absorption transfer rate in two-phase systems is important because it results in higher levels of dissolved oxygen, which, through rapid equilibrium partitioning, are then made available to cells in the aqueous phase.

CONCLUSIONS

This study has presented an improved framework for predicting oxygen absorption mass transfer coefficients in two-phase bioreactor systems as a function of the operating conditions. The proposed mechanistic model represents an improved methodology, in contrast to the previous empirical formulation, and corrects an oversight made in the analysis previously proposed by the authors (Nielsen et al., 2003). The current analysis remains reliant upon the assumption of equilibrium transfer of dissolved oxygen between the liquid phases of the two-phase system. This assumption is expected to be maintained under well-mixed conditions in which n-hexadecane constitutes the organic phase. It is important to acknowledge, however, that this may not be the case for other organic phases with greatly different physical attributes. Preliminary assessment of organic phase characteristics is recommended prior to application of the outlined approach to additional two-phase systems of interest.

Predictions made using the revised approach proposed in this paper, which have been verified by the presented data, are expected to be useful in future two-phase partitioning bioreactor modeling activities. The current, improved model of two-phase oxygen absorption clearly demonstrates that the addition of an organic phase with an affinity for dissolved oxygen can increase total rates of absorption in a bioreactor.

NOMENCLATURE

C_A	dissolved oxygen concentration in the aqueous phase (mg/L)
C_A^*	equilibrium dissolved oxygen concentration in the aqueous phase (mg/L)
D_I	impeller diameter (cm)
D_{OA}	organic-aqueous dissolved oxygen partitioning coefficient (mg/L organic/mg/L aqueous)
D_T	bioreactor vessel diameter (cm)
H_I	impeller height (cm)
$k_L a$	lumped two-phase volumetric oxygen mass transfer coefficient (h^{-1})
$k_L a_A$	lumped volumetric oxygen mass transfer coefficient the aqueous phase (h^{-1})
$k_L a_i$	lumped volumetric oxygen mass transfer coefficient in phase i (h^{-1})
$k_L a_O$	lumped volumetric oxygen mass transfer coefficient the organic phase (h^{-1})
$k_L a_{TP}$	lumped volumetric oxygen mass transfer coefficient in a two-phase, aqueous-organic dispersion (h^{-1})
$\dot{m}_A$	total rate of oxygen absorption into the single, aqueous phase (mg/h)
$\dot{m}_{TP}$	total rate of oxygen absorption into a two-phase dispersion (mg/h)
N	agitation rate (rpm)
P_g	power input to the aerated system (W)
t	time (h)
V	liquid working volume in the bioreactor (m^3)
V_A	aqueous volume (m^3)
V_O	organic volume (m^3)
V_T	total volume of the aqueous-organic dispersion (m^3)
v_s	superficial gas velocity (m/s)
w	volume-averaged dissolved oxygen weighting factor
X_{ORG}	organic phase volume fraction

Greek Letters

α, β, γ	nonlinear model parameters to be fit

Subscripts

A	aqueous phase
O	organic phase
TP	two-phase dispersion

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