A Novel Method of Simulating Oxygen Mass Transfer in Two-Phase Partitioning Bioreactors

David R. Nielsen, Andrew J. Daugulis, P. James McLellan

Department of Chemical Engineering, Queen's University, Kingston, Ontario K7L 3N6, Canada; telephone: 613-533-2784; fax: 613-533-6637; e-mail: daugulis@chee.queensu.ca

Received 15 December 2002; accepted 26 March 2003

Published online 23 June 2003 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/bit.10721

Abstract: An empirical correlation, based on conventional forms, has been developed to represent the oxygen mass transfer coefficient as a function of operating conditions and organic fraction in two-phase, aqueous–organic dispersions. Such dispersions are characteristic of two-phase partitioning bioreactors, which have found increasing application for the biodegradation of toxic substrates. In this work, a critical distinction is made between the oxygen mass transfer coefficient, k_La, and the oxygen mass transfer rate. With an increasing organic fraction, the mass transfer coefficient decreases, whereas the oxygen transfer rate is predicted to increase to an optimal value. Use of the correlation assumes that the two-phase dispersion behaves as a single homogeneous phase with physical properties equivalent to the weighted volume-averaged values of the phases. The addition of a second, immiscible liquid phase with a high solubility of oxygen to an aqueous medium increases the oxygen solubility of the system. It is the increase in oxygen solubility that provides the potential for oxygen mass transfer rate enhancement. For the case studied in which *n*-hexadecane is selected as the second liquid phase, additions of up to 33% organic volume lead to significant increases in oxygen mass transfer rate, with an optimal increase of 58.5% predicted using a 27% organic phase volume. For this system, the predicted oxygen mass transfer enhancements due to organic-phase addition are found to be insensitive to the other operating variables, suggesting that organic-phase addition is always a viable option for oxygen mass transfer rate enhancement. *Biotechnol Bioeng* **83:** 735–742, 2003.

Keywords: oxygen transfer; k_La; two-phase partitioning bioreactor

INTRODUCTION

Oxygen transfer limitations occur commonly in bioreactors, leading to decreased performance. When oxygen is limited, the metabolic rate of the microorganisms decreases significantly and the culture may respond adversely to the resulting stress (Lee, 1992). Numerous investigations have been performed to identify the effect of agitation and aeration on the rate of oxygen transfer, as evidenced by the large number of empirical formulations available in the literature. Some limited work has also been performed to observe the effect of the presence of a second liquid phase on oxygen mass transfer for organic compounds such as *n*-hexadecane (Hassan and Robinson, 1977; Ho et al., 1990; Ju and Ho, 1989; Zhao et al., 1999), *n*-dodecane (Hassan and Robinson, 1977), perfluorochemicals (McMillan and Wang, 1987, 1990), oleic acid (Linek and Benes, 1976), and vegetable oil (Zhao et al., 1999). Aqueous–organic dispersions have undergone increased application through two-phase partitioning bioreactors for the biodegradation of toxic and poorly water-soluble compounds, as evidenced by recent reviews (Deziel et al., 1999; Malinowski, 2001).

The limited solubility of oxygen in water is a physical constraint on bioreactor operation. The introduction of an immiscible organic solvent as a second liquid phase has the potential to aid in oxygen transfer in bioreactors. By adding an organic phase with a higher affinity for oxygen, larger amounts of oxygen are removed from the aeration gas stream, being retained in the two-phase system. Whereas no more than the saturation concentration of oxygen can be dissolved in either liquid phase, the supply of oxygen to the aqueous phase from the gas stream may be supplemented by equilibrium partitioning of dissolved oxygen from the organic phase to the aqueous phase. Provided that the partitioning mass transfer process is rapid, as was previously determined for *n*-hexadecane–water two-phase aerations (Zhao et al., 1999), the combined mechanisms will result in observably higher rates of oxygen transfer to the aqueous phase. Hassan and Robinson (1977) found that the addition of *n*-hexadecane to a dilute aqueous phase resulted in up to a 25% increase in oxygen transfer to the system. This effect was observed for experiments including 5% and 10% (v/v) *n*-hexadecane. Ho et al. (1990) also found that the addition of as little as 0.5% to 2% (v/v) *n*-hexadecane enhanced oxygen transfer enough to partially relieve oxygen limitations during fermentations.

Correspondence to: A. J. Daugulis

Contract grant sponsors: Natural Sciences and Engineering Research Council of Canada; Queen's University

Bioreactors will become oxygen-limited when the oxygen demand of the culture exceeds the oxygen supply rate to the bioreactor. According to two-film theory, the transfer rate of oxygen from the gas phase to the liquid phase may be represented by Eq. (1) (Shuler and Kargi, 2002):

$$\frac{dC}{dt} = k_L a \cdot (C^* - C) \tag{1}$$

where C represents the dissolved oxygen concentration in the aqueous phase, C^* represents the dissolved oxygen concentration in equilibrium with oxygen concentration in the gas phase (i.e., the saturation concentration of oxygen in the aqueous phase), and $k_L a$ represents the lumped volumetric mass transfer coefficient, the product of the mass transfer coefficient and the mass transfer area. The product $k_L a$ is often reported as a lumped parameter, because the two components are not easily separable experimentally. The saturation concentration of oxygen from air in water, C^*, is determined using Henry's Law [Eq. (2)], and can be calculated as 7.95 mg/L at 30°C:

$$C^* = \frac{[O_2]_{GAS}}{H} \tag{2}$$

Empirical correlations are available to predict the Henry's Law constant, H, as a function of temperature (Perry and Green, 1997). From Eq. (1), it is seen that oxygen transfer between the gas phase and the aqueous phase will continue until thermodynamic equilibrium is achieved, at which point the net transfer between the phases will become zero.

During typical operation, the rate of oxygen transfer can be increased with more aggressive agitation (greater power input) or higher aeration, as evidenced by Eq. (3), a typical correlation for estimating $k_L a$ values (Nielsen and Villadsen, 1994):

$$k_L a = \delta \cdot \left(\frac{P_g}{V}\right)^{\alpha} \cdot (v_s)^{\beta} \tag{3}$$

where δ, α, and β represent empirical constants; P_g represents the power requirement of the aerated bioreactor; V represents the bioreactor working volume; and v_s represents the superficial gas velocity through the bioreactor.

The present work was performed to obtain an empirical correlation to predict the rate of oxygen transfer to a bioreactor in the presence of an immiscible, organic liquid phase. The correlation is a function of the operating conditions of the bioreactor (aeration and agitation rates) as well as the organic content, and was created with the intention for use in simulating two-phase partitioning bioreactors to predict fermentation performance when using models that account for the effect of dissolved oxygen. Although numerous correlations are available to predict oxygen mass transfer as a function of operating conditions, none has been found that includes the effect of the presence of an organic phase.

MATERIALS AND METHODS

Range of Operating Conditions

Earlier studies have been conducted to measure oxygen mass transfer coefficients in two-phase systems. Of particular interest is the work of Hassan and Robinson (1977), who investigated an *n*-hexadecane–aqueous system similar to the one in this study. Using a similar reactor scale and operating conditions, $k_L a$ values were measured using the same dynamic gassing technique. Table I provides a comparison between conditions used in the present study and those used by Hassan and Robinson. Although the power input per unit volume and aeration rates are similar, the range of agitation rates used by Hassan and Robinson were much higher. Higher organic fractions, X_{ORG}, were chosen in this study to represent those used in previous two-phase fermentations (Yeom and Daugulis, 2001). In the absence of organic phase, Hassan and Robinson (1977) found $k_L a$ to range from 180 h^{-1} to 250 h^{-1} at 2 vvm (volume of air per volume reactor per minute) for power inputs of between 1000 and 5000 W/m^3.

Bioreactor Configuration

A total working volume of 1 L was chosen for use in a 2-L New Brunswick Bioflo I bioreactor (D_t = 11 cm). The two-phase liquid dispersion was agitated with two six-blade Rushton turbine impellers (D_i = 4.95 cm, H_i = 0.95 cm, 2.65-cm spacing), rotated on a 0.9-cm shaft, whereas four equally spaced baffles were used to enhance mixing. Air was supplied to the bioreactor with a standard New Brunswick Bioflo I sparger located at the base of the agitator shaft.

Liquid Phases

In the two-phase system investigated, the organic phase consisted of *n*-hexadecane (98% assay, Alfa Aesar). The aqueous phase was a typical growth medium for *Alcaligenes xylosoxidans* Y234 composed of 7 g/L $(NH_4)_2SO_4$, 0.75 g/L $MgSO_4 \cdot 7H_2O$, 2 g/L KH_2PO_4, and 0.075 mL of trace nutrient solution. This medium formulation is similar to that used in previous two-phase partitioning bioreactor degradation studies (Yeom and Daugulis, 2001). The aqueous medium was autoclaved in the bioreactor, whereas the organic

Table I. Comparison of experimental conditions used in this study and in previous work.

Operating variable	This study		Hassan and Robinson (1977)	
	Minimum	Maximum	Minimum	Maximum
P_g/V (W/m^3)	50	$9 \cdot 10^3$	$8 \cdot 10^2$	10^4
N (rps)	3.33	13.33	13.33	33
Q_g (vvm)	0.5	2.0	0.5	2.0
X_{ORG}	0	0.33	0	0.10

phase was autoclaved separately for later addition. Organic fractions of 0%, 10%, 25%, and 33% of the total working volume were used.

Analytical Procedure

The dissolved oxygen concentration was measured with a polarographic-membrane dissolved oxygen probe (D100 Series OxyProbe, Broadley and James Corp.) and monitored with a computer interface at 5-s intervals. Between runs, the bioreactor was de-aerated by sparging with high-purity compressed N_2 until only minimum levels of dissolved oxygen remained. At this point, air was diffused into the reactor until saturation was achieved. All experiments were conducted at 30°C.

Modeling

Simulations and nonlinear regression were performed with MATLAB software. The intrinsic function ODE15S was used for solving differential equations, whereas NLINFIT was used to estimate the parameters of the nonlinear model using the Gauss–Newton method.

RESULTS

The sampling frequency of the dissolved oxygen data acquisition system was rapid enough to provide numerous measurements of the dynamic response of the system to the step change in oxygen concentration of the diffused gas, as shown in Figure 1. Although the actual change in dissolved oxygen (DO) concentration is predicted to be a first-order absorption process, as represented in Eq. (1), the observed response is representative of a second-order process, indicative of the presence of probe-response dynamics. Probe-response dynamics must be accounted for when using polarographic membrane oxygen probes if the response time

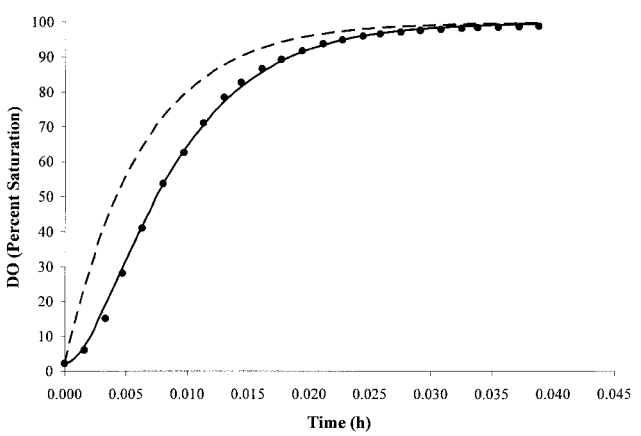

Figure 1. Typical dynamic response of the two-phase system to a change in oxygen concentration in the diffused air, including probe response at 600 rpm, 2 vvm, and 0% organic volume. (●) Experimental data; (—) second-order model, including probe response used to estimate k_La; (- - -) first-order model using the k_La estimate from the second-order model solution.

of the probe, $\tau_e = 1/k_e$, is of the same order of magnitude as $1/k_La$ (Ruchti et al., 1981). The response time of a membrane oxygen probe is typically determined as the time required for the output to reach 63.2% of the final value after an input step change (Badino et al., 2000; Tribe et al., 1995). Typical response times of sterilizable membrane oxygen probes range between 10 and 100 s (Badino et al., 2000). The response time of the D100 Series OxyProbe was estimated as 11.2 s at 30°C from literature values (Broadley and James Corp., 2000), and experimentally confirmed by our group. The lag in response of polarographic membrane oxygen probes can be dynamically simulated as oxygen diffusion through a thin membrane using Fick's Law (Hassan and Robinson, 1977; Heineken, 1970) or, more simply, with the use of a characteristic time constant (Badino et al., 2000; Ruchti et al., 1981; Tribe et al., 1995). Using the latter approach, the combined second-order response is modeled using Eq. (1), coupled with Eq. (4):

$$\frac{dC_e}{dt} = k_e \cdot (C - C_e) \tag{4}$$

The solution of the second-order model is shown in Figure 1, along with a typical set of data, and the predicted response for the same k_La value, neglecting probe dynamics. For operating conditions in which $k_La > 0.25 \cdot k_e$, the probe response was found to be an important consideration. Accounting for probe response yielded more accurate k_La estimates with values as much as 25% greater.

The measured values of k_La under different operating conditions are displayed in Figure 2, at different organic-phase fractions. The standard deviation of the experimental measurements was estimated to be 4 h^{-1}.

For aeration rates of <1.0 vvm, the effect of agitation rate was hardly noticed. Between 0.5 and 1.0 vvm there was nearly a tenfold increase in the observed mass transfer coefficient. Similarly, for agitation rates of <400 rpm, the mass transfer was relatively insensitive to the aeration rate. As the agitation rate was increased from 200 to 400 rpm, a nearly tenfold increase in k_La values was also observed. At all agitation rates, the Reynolds numbers suggest that the system was in the turbulent flow regime (Nielsen and Villadsen, 1994). However, at low agitation rates (200 rpm), the two liquid phases did not appear to be completely dispersed. In addition, large gas bubbles were observed in the two-phase experiments mixed at 200, and even 400 rpm, indicating that the gas and liquid phases were not well dispersed. Although liquid-phase mixing is crucial, the effect of the agitation rate on k_La values is also due to the shearing and dispersing of gas bubbles, which leads to increased gas–liquid mass transfer areas and contact times.

Interestingly, at 400 rpm the data collected during the experiments in the presence of an organic phase did not fit the characteristic form of the second-order model (see Fig. 1). This may have been due to the altered bulk fluid properties of the two-phase mixture. These results, together with the observed mixing behavior, suggest that a minimum agi-

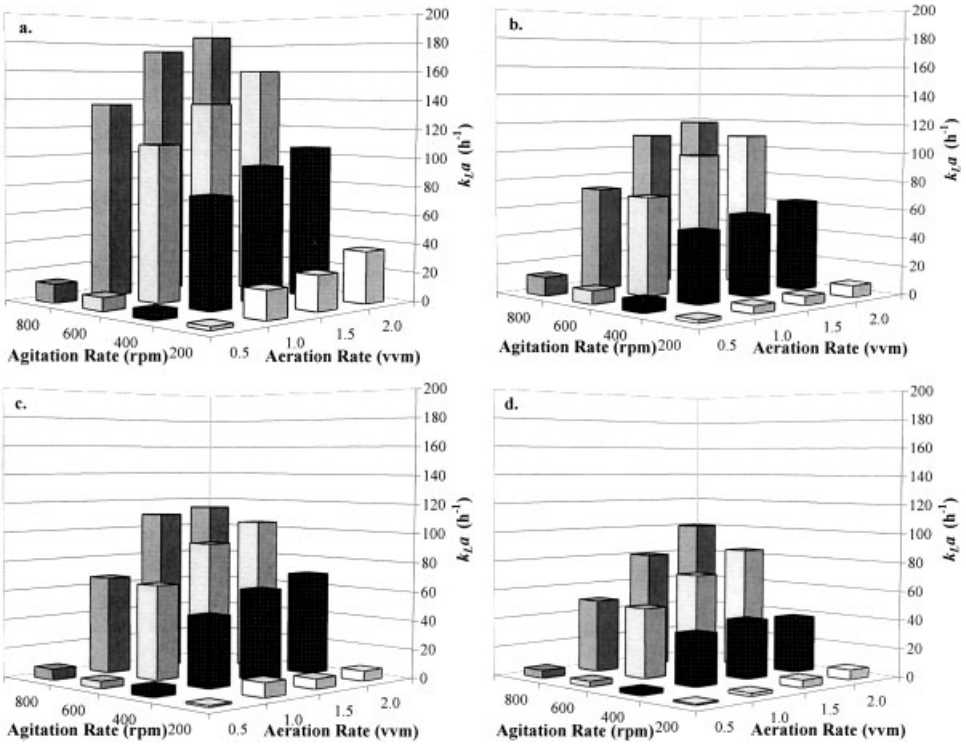

Figure 2. Experimental k_La values at various operating conditions and organic phase fractions of: (a) 0%; (b) 10%; (c) 25%; and (d) 33%.

tation rate of >400 rpm may be required to achieve sufficient mixture in these two-phase dispersions. Maintaining dispersions in aqueous–organic mixtures has been a problematic issue in the past. While operating a two-phase partitioning bioreactor with an *n*-hexadecane organic fraction of 0.33 and a 1.5-L total working volume to degrade benzene, Yeom and Daugulis (2001) found that the two liquid phases could remain distinct for agitation rates of up to 500 rpm when aerated at 0.25 vvm.

The results in Figure 2 were used to develop an empirical correlation to predict k_La values as a function of operating conditions and organic-phase content. Figure 2 clearly indicates that the experimental value of k_La depends on the organic fraction present; more specifically, as the organic fraction in the system increased, the value of k_La decreased. Therefore, an expression that accounts for the amount of organic fraction is necessary to represent the data. Using Eq. (3) as a reference, the simplest model for representation of the data was:

$$k_La = \delta \cdot \left(\frac{P_g}{V}\right)^{\alpha} \cdot (v_s)^{\beta} \cdot (1 - X_{\mathrm{ORG}})^{\gamma} \qquad (5)$$

where P_g, V, and v_s are as described earlier; X_{ORG} is the fraction of the total bioreactor working volume that is in the organic phase; and δ, α, β, and γ represent empirical constants that must be estimated. The units for the operating conditions used in Eq. (5) are as follows: P_g in watts, V in square meters, v_s in meters per second, and X_{ORG} is the organic volume fraction. Using least-squares nonlinear regression employing the Gauss–Newton method, parameters δ, α, β, and γ in Eq. (5) were estimated to be 650, 0.31, 0.70, and 1.70, respectively. Using the empirical correlation given by Eq. (3) for single-phase absorptions, Moo-Young and Blanch (1981) suggested typical values of α and β as 0.7 and 0.3, respectively, for noncoalescing media and 0.4 and 0.5, respectively, for coalescing medium, when mechanically agitated with a six flat-bladed disk turbine. These values indicate that the functional dependence of k_La on operating conditions for the two-phase system was slightly different from that of single-phase systems.

The model appears to provide a good fit to the data, as evidenced in Figure 3, showing predicted versus experimental results with a line of slope equal to 1. Although most of the predicted values appeared to be well distributed about the experimental data, the model did not represent the group of data obtained at low k_La values very well. For operating conditions that produce low k_La values, the model systematically overpredicts the value of the oxygen mass transfer coefficient. As mentioned previously, at the low end of the operating conditions, the response of the system to the inputs was weak, and the behavior was not representative of the entire range of operating conditions. Operating conditions producing k_La values in the low range are used less commonly as they will often lead to oxygen limitations during fermentations. To improve the estimates at the low end of operating conditions, the operating space could have been separated into two distinct regions and two sets of parameter values could have been estimated for Eq. (5), one

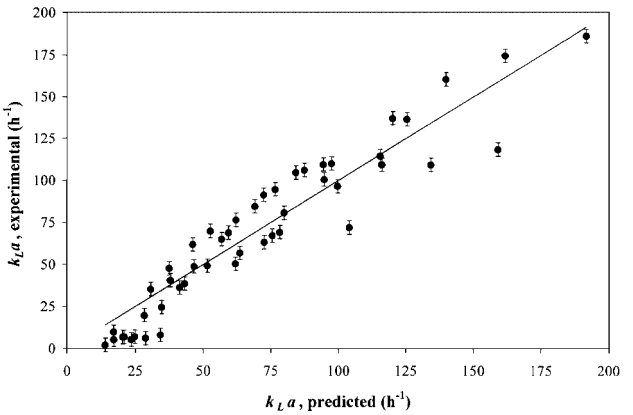

Figure 3. Experimental versus predicted $k_L a$ values using Eq. (5) and estimated parameters. Experimentally determined error bars at one standard deviation, 4 h^{-1}.

for use in each region. This was not done, however, as we intended to develop a single correlation for use over the entire feasible operating space.

The model structure used in Eq. (5) was based on conventional models so that parameter estimates associated with the operating variables could be compared with previous correlations. Because the model form can have a strong influence on the fit to the data, indications of lack of fit may suggest problems with the choice of model form. Fortunately, residual analysis did not suggest that the model is lacking in any specific functionality, or that this model was misapplied to the data (residual analysis not shown). For low $k_L a$ values, additional terms could be included in the model to improve the estimation of these data. However, because the response of these data to the operating variables is misrepresentative of the typical operating range, such additional model terms would be unnecessary for most applications.

DISCUSSION

Figure 2 indicates that, in general, as the organic fraction in the bioreactor was increased, the experimental value of $k_L a$ decreased. This is contrary to what was originally anticipated, and does not support the observation of enhanced oxygen transfer in fermentations containing a second immiscible liquid phase. For "gas-in, gas-out" dynamic oxygen transfer experiments, the obvious result of having a greater oxygen capacity in the liquid phase is that the system will require greater amounts of oxygen to saturate. This means that, for a fixed inlet gas oxygen mass flow, systems with greater organic phase content will require longer aeration times to saturate. The result is lower experimental $k_L a$ values. Referring to Eq. (1), enhanced oxygen mass transfer is due to an increase in dC/dt, which can result from an increase in $k_L a$ or an increase in the driving force term, $(C^* - C)$. The model developed here assumes that the two-phase dispersion behaves as a single, homogeneous phase with bulk fluid properties estimated as the volume-averaged weighted values of each phase. From the solubility data, the partitioning coefficient for dissolved oxygen between *n*-hexadecane and a sodium sulfite aqueous solution was reported to be 7.52 at 30°C by Hassan and Robinson (1977), and 8.2 at 22°C between *n*-hexadecane and water by Ju and Ho (1989). Ho et al. (1990) also found the solubility of oxygen in *n*-hexadecane to be "more than 8 times higher than in pure water." Because the organic phase has been shown to have a higher oxygen solubility than the aqueous phase, when greater organic fractions are added to the bioreactor the oxygen absorption capacity of the system increases. Using a polarographic membrane-dissolved oxygen probe, it is not possible to distinguish between the different saturation concentrations that exist in each phase as a function of organic fraction, because the probe measures the tension of dissolved oxygen and not the actual concentration. At equilibrium, the dissolved oxygen tension in the two liquid phases and the gas phase are equivalent, regardless of phase volumes (Hassan and Robinson, 1977).

The difference in oxygen solubility between the two liquid phases is the driving force for partitioning mass transfer. For a two-phase dispersion, the bulk oxygen saturation concentration can be estimated as the volume average of the saturation concentrations of oxygen in water and *n*-hexadecane, $C^*_{\text{vol avg}}$, as previously assumed by Ho et al. (1990) and Linek and Benes (1976). With this assumption, estimated values of the concentration weighting factor, w, based on the volume-averaged oxygen saturation concentrations in the two-phase dispersions, are listed Table II. When predicting the actual rate of oxygen mass transfer in a two-phase dispersion, we propose that the difference in saturation concentration between the phases must be accounted for because the two liquid phases are in equilibrium. Eq. (1) must be scaled by the factor w to account for the dissolved oxygen solubility difference in two-phase dispersions, as follows:

$$\frac{dC}{dt} = k_L a \cdot w \cdot (C^* - C) \tag{6}$$

Similar expressions have been developed for two-phase dispersions of water and *n*-alkanes (Linek and Benes, 1976). With the assumption of increased bulk oxygen solubility, the difference in oxygen solubilities between the two liquid phases is accounted for and the model is able to predict the

Table II. Concentration weighting factors based on volume-averaged oxygen saturation concentrations of two-phase dispersions at 30°C.

X_{ORG}	$w = C^*_{\text{vol avg.}}/C^*$
0.00	1.00
0.10	1.65
0.25	2.63
0.33	3.15

improved oxygen transfer as a result of the presence of the organic phase using data from aeration experiments (Fig. 4).

When Eqs. (5) and (6) are combined to predict oxygen mass transfer rates in the *n*-hexadecane–aqueous two-phase system, the model predicts the presence of a maximum value of the rate of mass transfer, because, as the organic fraction increases, w increases more slowly than k_La decreases. As seen in Figure 5, for the *n*-hexadecane–water system, the predicted optimal organic fraction, $X_{ORG,\ optimal}$, for oxygen transfer is 0.27, corresponding to a 58.5% increase in oxygen mass transfer, relative to single-phase aqueous aeration. The effect of the organic phase on the rate of mass transfer of oxygen to the liquid is further illustrated in Table III, where the predicted percent increase in oxygen transfer rates with respect to the single liquid-phase aqueous aeration are compared. Because v_s is independent of X_{ORG}, and P_g is a very weak function of X_{ORG} (for the organic fractions studied), for any constant combination of agitation and aeration rates, it is predicted that the addition of organic phase will lead to an increased oxygen mass transfer rate as determined by the dependence illustrated in Figure 5. This means that the predicted percent increase in oxygen mass transfer rates as a result of the presence of an organic phase is independent of the other operating conditions. Thus, the predicted enhancement to oxygen mass transfer rates shown in Figure 5 and Table III would hold equally true at 600 rpm and 1 vvm as they would at 800 rpm and 2 vvm, for example. This is not to say that the actual oxygen mass transfer rates would be equivalent under these two different sets of conditions, but rather that the enhancing effect of the organic phase would be equivalent. This is analogous to stating that the functional dependence of the two-phase oxygen mass transfer coefficient on the agitation and aeration rates is identical to the dependence of a single, aqueous-phase oxygen mass transfer coefficient for the same operating conditions, a conclusion also found experimentally by Hassan and Robinson (1977).

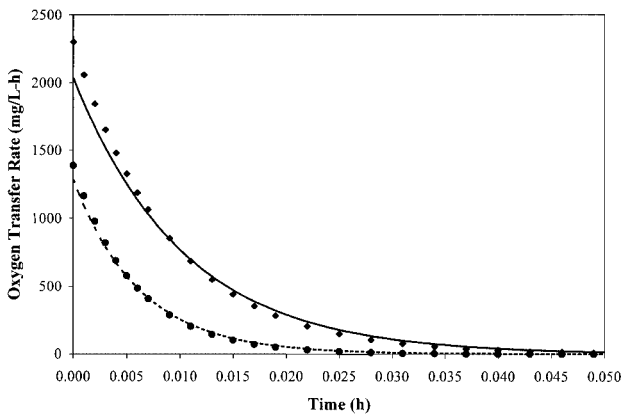

Figure 4. Comparison of experimental and predicted oxygen mass transfer rates for different organic-phase fractions at 800 rpm and 1 vvm. (♦) Experimental data at 25% organic phase; (—) predicted data at 25% organic phase; (- - -) experimental data at 0% organic phase; and (●) predicted data at 0% organic phase.

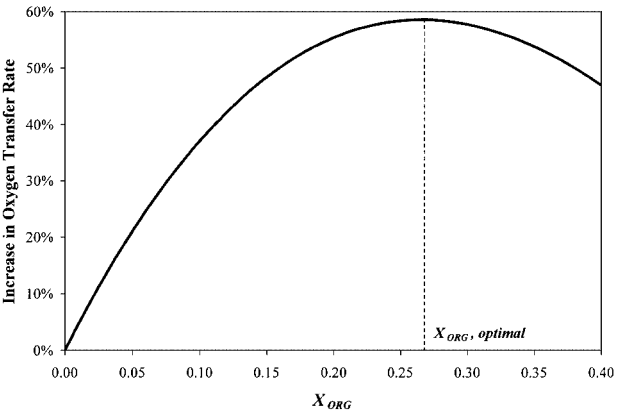

Figure 5. Identification of the optimal organic-phase fraction in the *n*-hexadecane–aqueous two-phase system.

Combining the oxygen-transfer data of Linek and Benes (1976) with their proposed two-phase oxygen transfer mechanism, the addition of 0.10 and 0.15 volume fractions of *n*-alkanes enhanced the rates of oxygen transfer by 8.1% and 21.4%, respectively. Hassan and Robinson (1977) observed that the addition of *n*-hexadecane in volume fractions up to 0.10 resulted in an enhancement of oxygen transfer by up to 25%. In their study, however, the improved oxygen transfer was accounted for in the reported values of k_La, rather than the enhanced dissolved oxygen saturation of the two-phase system. In this study we have shown that improved oxygen transfer rates are a consequence of the increased oxygen solubility of the dispersion and, in fact, experimental mass transfer coefficients were found to decrease with increasing organic fraction. No empirical correlation relating k_La to the operating conditions and organic fraction was developed in the Hassan and Robinson investigation.

It is clear from Figure 5 that enhancements to oxygen mass transfer rates have been made as a result of the introduction of an organic phase, and that an optimal organic fraction exists for maximizing the rate of oxygen mass transfer to the system. However, the potential for oxygen mass transfer rate enhancements as a result of organic-phase addition will depend strongly on the physical properties of that phase. Because w is a function of the oxygen partitioning coefficient between the two liquid phases, it will be unique for each organic phase. The functional dependence of k_La on X_{ORG} will also depend on the identity of the organic phase. This may mean that parameter γ of Eq. (5) will simply require re-estimation for each organic phase.

Table III. Predicted increase in oxygen mass transfer rates at the organic contents studied.

Organic fraction			
0	0.10	0.25	0.33
0%	37.1%	58.4%	55.7%

However, it is also possible there may exist organic phases for which the form of Eq. (5) is inadequate to represent the mass transfer coefficient as a function of organic content, and will require the development of alternative model forms.

This study has focused only on increasing oxygen transfer through organic phase addition, but there are several other considerations that must be made. Increasing organic content can carry high costs, particularly for expensive organic compounds. Also, as the organic volume fraction is increased, the aqueous volume fraction decreases. Because the aqueous phase is where biological reactions take place, decreasing the aqueous phase volume will decrease the overall productivity of the process. The potential for improved fermentation performance as a result of organic phase addition is therefore very application-specific.

CONCLUSIONS

A novel means for representing oxygen mass transfer in two-liquid-phase bioreactor systems has been proposed. The enhanced oxygen mass transfer rate in aqueous–organic dispersions has been properly accounted for by crediting both the functional dependence of k_La on the organic fraction and the enhanced solubility of oxygen in an aqueous–organic dispersion. The empirical correlation developed to predict the values of k_La, under different conditions of agitation rate, aeration rate, and organic phase content, is a useful tool for simulating oxygen transfer in a two-phase partitioning bioreactor and, ultimately, its effect on fermentation performance in cell-based systems.

Oxygen transfer rates have been shown to be enhanced due to the presence of an immiscible organic phase with high oxygen solubility. Furthermore, there exists an optimal organic fraction that maximizes the oxygen mass transfer enhancement under all sets of conditions. Operating policies for two-phase fermentations can be tailored to capitalize on this effect. The predicted oxygen mass transfer rate enhancements resulting from organic-phase addition were found to be insensitive to the other operating variables. This suggests that organic-phase addition is always an option for enhancing oxygen mass transfer rates, even when other operating variables are confined or their effect has been exhausted.

This work has focused on developing a novel means of describing the overall mass transfer rate of oxygen from the gas phase to a homogeneous, aqueous—organic two-phase dispersion and its functional dependence on the operating conditions. Insufficient data were collected to estimate the individual mass transfer coefficients from the gas phase to each of the two liquid phases, or their individual responses to changes in operating conditions. Conducting further experiments to obtain these individual mass transfer coefficients would be of great value in gaining further insight into the constitutive mass transfer phenomena of an aqueous–organic dispersion, and also to assess the adequacy of the homogeneous model. Future studies should include chemical analysis to verify that the increased bulk oxygen solubility due to organic-phase presence can be represented adequately by weighted volume-averaged solubility, or by another suitable means of describing the difference. With improved knowledge of this phenomenon, the actual mass transfer of oxygen to the two-phase system can be predicted with greater confidence, and will be used in our modeling activities to predict the performance of two-phase partitioning bioreactor systems.

NOMENCLATURE

C	aqueous dissolved oxygen concentration (mg/L)
C^*	equilibrium aqueous dissolved oxygen concentration (mg/L)
$C^*_{\text{vol avg}}$	volume-averaged dissolved oxygen concentration (mg/L)
C_e	electrode-measured dissolved oxygen concentration (mg/L)
C_{GAS}	gas-phase oxygen concentration (mg/L)
C_0	initial aqueous dissolved oxygen concentration (mg/L)
D_i	impeller diameter (cm)
D_t	bioreactor vessel diameter (cm)
H	Henry's Law constant for oxygen between the gas and aqueous phases (mg/mg)
H_i	impeller height (cm)
k_e	electrode time constant (h^{-1})
k_La	lumped oxygen volumetric mass transfer coefficient (h^{-1})
N	agitation rate (rpm)
P_g	power input to the aerated system (W)
Q_g	aeration rate (vvm)
t	time (h)
t_0	initial time (h)
V	liquid volume of the bioreactor (m^3)
v_s	superficial gas velocity (m/s)
w	volume-averaged dissolved oxygen weighting factor
X_{ORG}	organic fraction
$X_{\text{ORG, optimal}}$	optimal organic fraction

Greek letters

$\delta,\alpha,\beta,\gamma$	nonlinear model parameters to be fit
τ_e	electrode response time (s)

References

Badino AC, Facciotti MCR, Schmidell W. 2000. Improving k_La determination in fungal fermentation, taking into account electrode response time. J Chem Technol Biotechnol 75:469–474.

Broadley and James model D100 series OxyProbe instruction manual. P1806 Rev. B. Irvine, CA: Broadley and James Corp.

Deziel E, Comeau Y, Villemur R. 1999. Two-liquid-phase bioreactors for enhanced degradation of hydrophobic/toxic compounds. Biodegradation 10:219–233.

Hassan ITM, Robinson CW. 1977. Oxygen transfer in mechanically agitated aqueous systems containing dispersed hydrocarbon. Biotechnol Bioeng 19:661–682.

Ho CS, Ju LK, Baddour RF. 1990. Enhancing penicillin fermentations by increased oxygen solubility through the addition of *n*-hexadecane. Biotechnol Bioeng 36:1110–1118.

Heineken, FG. 1970. On the use of fast-response dissolved oxygen probes for oxygen transfer studies. Biotechnol Bioeng 12:145–154.

Ju LK, Ho CS. 1989. Oxygen diffusion coefficient and solubility an *n*-hexadecane. Biotechnol Bioeng 34:1221–1224.

Lee JM. 1992. Biochemical engineering. Englewood Cliffs, NJ: Prentice-Hall. p 240.

Linek V, Benes P. 1976. A study of the mechanisms of gas absorption into oil–water emulsions. Chem Eng Sci 31:1037–1046.

Malinowski JJ. 2001. Two-phase partitioning bioreactors in fermentation technology. Biotechnol Adv 19:525–538.

McMillan JD, Wang DIC. 1987. Enhanced oxygen transfer using oil-in-water dispersions. Biochemical engineering V. Ann NY Acad Sci 506:569–582.

McMillan JD, Wang DIC. 1990. Mechanisms of oxygen transfer enhancement during submerged cultivation in perfluorochemical-in-water dispersions. Biochemical engineering VI. Ann NY Acad Sci 589: 283–300.

Moo-Young M, Blanch HW. 1981. Design of biochemical reactors. Mass transfer criteria for simple and complex systems. Adv Biochem Eng 19:1–69.

Nielsen J, Villadsen J. 1994. Bioreaction engineering principles. New York: Plenum Press. p 295–342.

Perry RH, Green, DW. 1997. Perry's chemical engineers' handbook, 7th ed. New York: McGraw-Hill. p 2–127.

Ruchti G, Dunn IJ, Bourne JR. 1981. Comparison of dynamic oxygen electrode methods for the measurement of k_La. Biotechnol Bioeng 23:277–290.

Shuler ML, Kargi F. 2002. Bioprocess engineering: basic concepts, 2nd ed. Englewood Cliffs, NJ: Prentice-Hall. p 171.

Tribe LA, Briens CL, Margaritis A. 1995. Determination of volumetric mass transfer coefficient (k_La) using the dynamic "gas out-gas in" method: analysis of errors caused by dissolved oxygen probes. Biotechnol Bioeng 46:388–392.

Yeom SH, Daugulis AJ. 2001. Development of a novel bioreactor system for treatment of gaseous benzene. Biotechnol Bioeng 72:156–165.

Zhao S, Kuttuva SG, Ju LK. 1999. Oxygen transfer characteristics of multiple-phase dispersions simulating water-in-oil xanthan fermentations. Bioproc Eng 20:313–323.