Quick Procedure to Evaluate the Oxygen Mass Transfer Resistance in Aerated Laboratory-scale Bioreactors

MANUELA DIANA ENE[1] , IULIANA JIPA[2], GHEORGHE MARIA[2,*], ANICUTA STOICA-GUZUN[2], MARTA STROESCU[2]

[1] Biotehnos Comp., 3-5 Gorunului Str., Otopeni, Romania

[2] University Politehnica of Bucharest, Department of Chemical Engineering,, 1-7 Gh. Polizu Str., 011061, Bucharest, Romania

Evaluation of the mass transfer resistance in aerated, well-mixed, laboratory-scale bioreactors, and of the overall gas-liquid mass transfer coefficient K_La is essential in any further kinetic and optimization analysis of a bioprocess. This is the case of enzymatic oxidation reactions or bioprocesses involving living cell cultures, thus allowing distinguishing between kinetic and transporting terms in the mass balance equations. A quick and simple experimental procedure, conducted in the absence of any reaction, and involving repeated water de-aeration (even if not complete) by sparging, using compressed N_2 and re-aeration, leads to evaluate the K_La coefficient from the dissolved oxygen recorded kinetic curves. Comparatively to the classical approach, the procedure can be applied without changing the reaction conditions and liquid composition. The derived coefficient can be then correlated with the mixing and aeration conditions, allowing the possibility to choose the optimal aeration regime. As the volumetric mass transfer coefficient is dependent on the system characteristics and of a large number of factors, such a quick methodology is preferred to replace an approximate value taken from literature as it allows an easy implementation on any commercial bench-scale bioreactor and it may be carried out before starting the bioreaction.

Keywords: oxygen-water mass transfer coefficient, bioreactor

Oxygen transfer intensity is a major concern whenever conducting enzymatic reactions or bioprocesses, which require air for microbial growth or enzymatic oxidative steps. Agitation in bio-reaction units is directly related to oxygen transport from the gas phase to liquid phase followed by the oxygen uptake by the individual microbial cell or oxygen consumption in enzymatic oxidation reactions. In fact, activity of microorganisms or of the enzyme (oxidase) is monitored by the use of oxygen from supplied air. A limitation in the supplied oxygen, due to mass transport resistance or a consumption rate faster than the transported oxygen rate, may cause a decrease in the cell growing rate or a decrease of the overall enzymatic reaction rate. Consequently, a close control of the available oxygen in the liquid phase is implemented for any type of aerobic bioreactor, the amount of dissolved oxygen (DO) being continuously measured by means of DO-meters.

As the solubility of oxygen in water is not very high (ca. 9 mg/L at 20°C), its overall consumption rate is dependent on a large number of factors, the most important being the diffusion coefficient, temperature, gas-liquid mass transfer coefficient K_La , and the rate of microbial/enzymatic reactions. Oxygen transfer from gas phase to the reaction site (cells, enzyme) takes place in several steps. First, oxygen is transferred through the gas-liquid interface, then it is transported through bulk liquid and finally into the microbial cell. To improve the oxygenation rate, sophisticated mixing and air sparger systems are implemented on both laboratory and industrial scale bioreactors. Air under pressure is supplied through a tube end consisting in 'O' rings with very fine holes or orifices. The size of bubbles, which affects the mass transfer process, depends on the holes' size and type of sparger. For very fine and uniform bubbles with effective gas dispersion, a micro-sparger system is used instead of a sparger, consisting in highly porous ceramic material. Air dispersion in liquid phase is not only related to the sparger, but also on the type of impeller and mixing intensity. The appropriate position and type of the impeller can ensure the even distribution of the gas in the reactor. High agitation is favourable to the mixing, but a very high stirring speed may cause shear forces, damaging the cells and leading to a spotty aeration of the liquid.

Special chapters are dedicated to mass transfer evaluation in the framework of bioreactor design and operation with various areas of applicability: industrial biosynthesis [1-5]; biological treatment of wastewaters [6-7,20]; oxidative treatment of dissolved inorganic substances [6], etc.

To optimise the aeration rate, knowing the gas-liquid mass transfer resistance is essential not only for a theoretical process analysis, but also for practical reasons related to bioprocess development. As the experimental observation can indicate only the overall oxygen uptake by the bioprocess, it is highly important to separate the contribution of the physical gas-liquid transport to its consumption in bio-reactions. Such analysis is possible only from separate determinations of the K_La coefficient and of the (bio)reaction rates. Experiments should be conducted in the absence of reaction, or under operating regimes at high aeration rates, making the whole process kinetically controllable.

The volumetric mass transfer coefficient K_La is dependent on a large number of factors. This is why its precise evaluation is difficult due to its strong dependence on the liquid phase properties, mixing, gas solubility, operating conditions (temperature), sparger depth, aeration rate, vessel volume and geometry, baffles, liquid surface tension, etc. [8]. The empirical correlations from literature are dependent on each studied system and several corrections are applied when moving to another system

* email: gmaria99m@hotmail.com

[1,5-7]. This is why such a $K_L a$-correlation can usually offer only rough prediction of the mass transfer coefficient, making further bioprocess kinetic identification and optimization analysis questionable.

On the other hand, developing a precise correlation of $K_L a$ with various factors involves steady experiments to determine separate influences of independent variables on the oxygen mass transport, under variable operating conditions and at various scales of the gas-liquid system.

The aim of this paper is to describe a quick and simple experimental procedure to precisely evaluate the $K_L a$ coefficient, in a lab-scale bioreactor. Experiments are conducted in the absence of any reaction, and involve repeated water de-aeration (using nitrogen) and re-oxygenation, leading to measure the DO kinetic curves and eventually to determine the $K_L a$ value. The derived coefficient can be then correlated with the mixing and aeration conditions, allowing to achieve the optimal operating conditions. As $K_L a$ is dependent on each system characteristics, such a procedure is preferred to the correlations from literature, due to its easy applicability and increased precision in further analysis of the bioprocess.

Experimental part

The experiments were carried out in a 2 L working volume laboratory bioreactor (Biostat A Plus, Sartorius) presented in figure 1, with computer-controlled and recorded parameters (temperature, *p*H, DO, liquid-level). The six-blade disk impeller and baffles ensures a satisfactory homogenisation of the bioreactor content, while the air micro-sparger system ensures a good gas-liquid contact, the bubble size being dependent on the aeration rate.

The classical approach to evaluate the oxygen mass transfer resistance from the gas bubbles to bulk liquid, is to use clean water in the absence of any (bio)chemical reaction and, initially to remove dissolved oxygen from a known volume of water by addition of sodium sulphite [7]. Then, the water is re-oxygenated near the saturation level, by measuring the dissolved oxygen concentration (DO) through time during the re-aeration period at several different points, to better represent the tank content. Based on the [DO](t) kinetic curve, the apparent volumetric coefficient $K_L a$ is estimated from a simplified mass transfer model of oxygen (see below), while the equilibrium saturation concentration results from the DO value obtained as the aeration period approaches infinity. However, this procedure suffers from several disadvantages, the tested reactor content being different to the bio-reaction environment (to prevent its contamination), while the used sulphite may change the characteristics of the liquid phase.

In the present study, the used procedure is a non-invasive one, consisting in partial removal of oxygen from liquid phase by first sparging nitrogen until a significant reduction of DO-level is realized. In the next step, the liquid is re-aerated until saturation using compressed air introduced through the ring sparger, and monitoring the increase in the DO concentration. The quick $K_L a$ estimation can be done witout complete removal of the oxygen from liquid (i.e. around 0% oxygen in water), the missing first part of the aeration curve being of minor importance for such a purpose.

Repeated experiments under various operating conditions allow the correlation of the $K_L a$ coefficient with different aeration and mixing rates. Such a procedure is easily implementable and does not raise special equipment for a commercial bioreactor. Besides, the inert nitrogen does not change the characteristics of the liquid-phase, experiments being conducted before starting the systematic study of the biochemical/biological process.

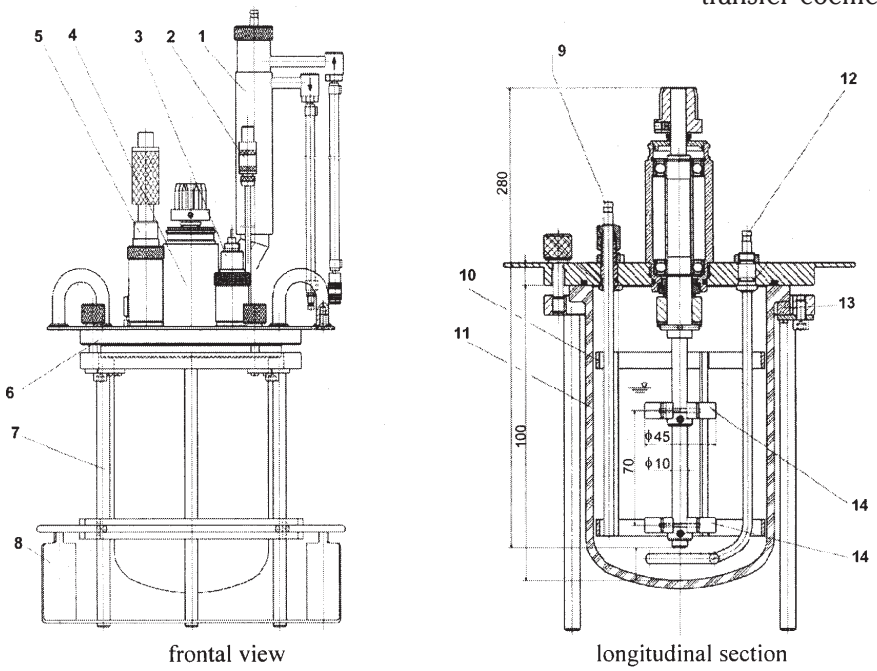
Results and discussions

The demonstrative experiments have been conducted at 30°C using 1 L distilled water, by checking aeration rates in the range of 0.4-1.4 L min^{-1} (i.e. a superficial gas velocity of 0.5-1.7 . 10^{-3} m/s), under various mixing conditions given by stirrer speeds in the range of 200-400 rpm. After de-aeration of the liquid by sparging nitrogen, each recorded oxygen concentration curve [DO](t) was plotted and used to evaluate the volumetric mass transfer coefficient (figs. 2-3).

Oxygen transfer at low concentrations and stationary conditions is proportional to the oxygen concentration gradient existing on the interface of the gas (bubbles) and liquid bulk phase [1]:

$$N = k_L (c_i - c_L) , \qquad (1)$$

where: N= the oxygen flux in mg m^{-2} s^{-1}; k_L= partial mass transfer coefficient on liquid film, in m s^{-1}; c_i= oxygen



frontal view longitudinal section

Fig. 1. Batch-scale bioreactor
1- exhaust cooler; 2- temperature sensor; 3- pH electrode; 4- stirrer; 5- pO_2 electrode; 6- reaction vessel; 7- vessel stand; 8- bottle support; 9 - sampling tube; 10 - baffles; 11 - reaction vessel; 12 - ring sparger; 13 - support; 14 - six blade disk impeller

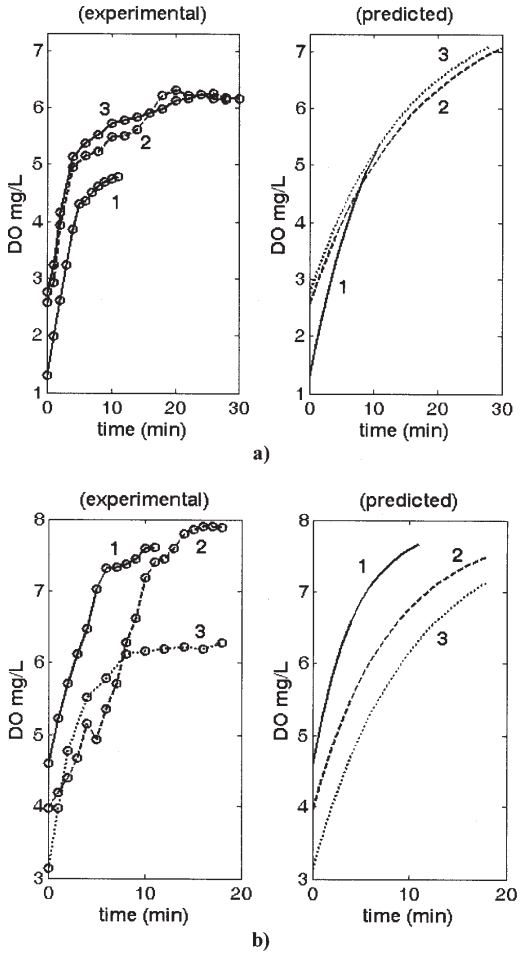


Fig. 2. Experimental (-O-) and predicted (simple lines) oxygenation kinetic curves of distilled water at 30°C, by using N_a = 200 rpm (a), and N_a = 300 rpm (b). The curves correspond to the aeration flow-rates of: (a) (1)= 1.4 L min^{-1}, (2)= 0.6 L min^{-1}, (3)= 0.4 L min^{-1} ; (b) (1)= 1.0 L min^{-1}, (2)= 0.6 L min^{-1}, (3)= 0.4 L min^{-1} .

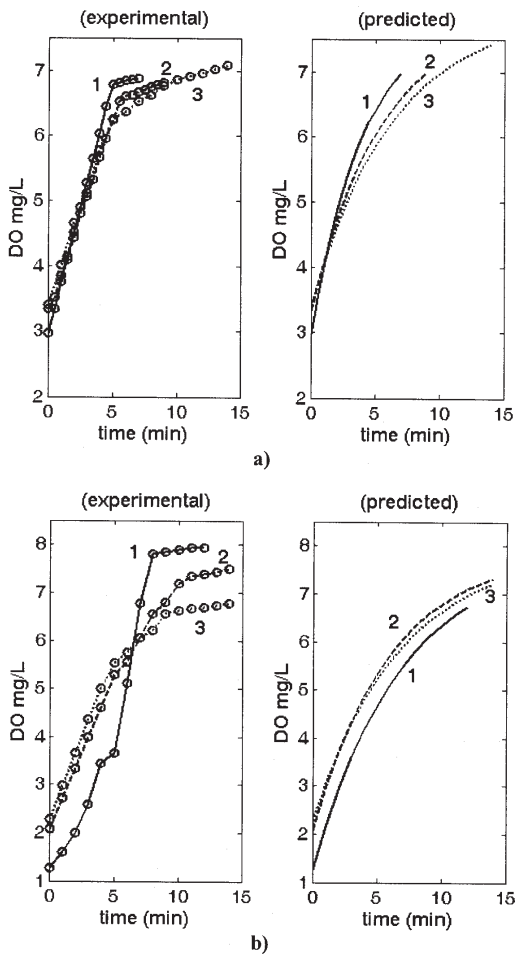


Fig. 3. Experimental (-O-) and predicted (simple lines) oxygenation kinetic curves of distilled water at 30°C, by using N_a = 400 rpm (a), and N_a= 500 rpm (b). The curves correspond to the aeration flow-rates of (1)= 1.0 L min^{-1}, (2)= 0.6 L min^{-1}, (3)= 0.4 L min^{-1}

concentration at the gas-liquid interface, in mg m^{-3}; c_L = oxygen concentration in the liquid phase (assumed to be homogeneous), in mg m^{-3}. On the other hand, the stationary mass flux of oxygen in the gas phase to liquid phase is stated as:

$$N = k_g (p_{O_2} - p_{O_2,i}) , \qquad (2)$$

where: k_g = partial mass transfer coefficient at the gas film, in mg m^{-2} s^{-1} atm^{-1}; p_{O2} = oxygen partial pressure in the gas phase (bubbles), in atm; p_{O2},i = oxygen partial pressure at the interface, in atm. If Henry's law is assumed to be fulfilled at the interface, one can write:

$$p_{O_2} = H\, c_L^* , \qquad (3)$$

where c^*_L = oxygen saturation (equilibrium) concentration in liquid phase, in mg m^{-3}; H = Henry's constant for oxygen at the experimental conditions, in atm mg^{-1} m^3, then by combining eqs. (1)-(3) one obtains:

$$N = K_L (c_L^* - c_L) \; ; \; \frac{1}{K_L} = \frac{1}{k_L} + \frac{1}{Hk_g} , \qquad (4)$$

where K_L = overall mass transfer coefficient in liquid phase in m s^{-1}. For the oxygen transfer rate (denoted by r_{O2}), the interface area plays an important role. If one assumes air bubbles as being on spherical-shape, the surface area is defined by [9]:

$$a = \frac{surface\ area\ of\ bubbles}{volume} = \frac{6\,\varepsilon_g}{d_b} , (m^{-1}) \qquad (5)$$

where ε_g = gas holdup; d_b = mean bubble diameter, in m. By multiplying the both sides of eq. (4) with a, the following mass transfer rate results:

$$-r_{O_2} = \frac{dc_L}{dt} = (K_L a)(c_L^* - c_L) . \qquad (6)$$

Reported values of $K_L a$ [9-10] reveal that, for most systems, the whole process is controlled by the liquid-phase transfer, $k_L a$ being with several orders of magnitude smaller than the $k_g a$ (usually 1-3 s^{-1}).

By integrating eq. (6) under isothermal (iso-pH, stationary operating conditions), one obtains the analytical form of the aeration curve $c_L(t)$ (i.e. the DO evolution in liquid), and an estimate of the constant $K_L a$ coefficient:

$$ln\frac{c_L^* - c_L}{c_L^* - c_{L,0}} = -(K_L a)t \;\Rightarrow\; K_L a = \frac{1}{t} ln\frac{c_L^* - c_{L,0}}{c_L^* - c_L} , \qquad (7)$$

where $c_{L,0}$ = initial concentration of oxygen in liquid when aeration starts, in mg m^{-3}; C_L = DO concentration at time t.

Using sparger air in bioreactor, the saturation value c^*_L at 30°C depends on the distilled water quality, being of ca. 7.40-7.95 mg L^{-1} (for = 845.13 atm mol^{-1} L) [6,11]. Using the recorded values of $c_L(t)$ from figures 2-3, the $K_L a$ coefficient was evaluated by averaging the values estimated with eq. (7) over the whole kinetic aeration

curve, under specified aeration and mixing conditions. The obtained results are in the range of K_La = 0.00060.0102 s^{-1}. These results are in a fair agreement with data from literature, that is: K_La= 0.020 s^{-1} for a small bioreactor (10 mL volume, 25°C, pure oxygen flow [12-14]); K_La = 0.001-0.040 s^{-1} in the presence of mycelial aggregates of fungus biomass in liquid phase (4 L volume, 21°C, air flow, N_a = 200-600 rpm, superficial gas velocity of 1-5 . 10^{-3} m s^{-1} [15]); K_La= 0.007-0.044 s^{-1} in the presence of surfactants and oil in liquid phase (1.7 L volume, two-phase liquid dispersion [16]); K_La = 0.001-0.004 s^{-1} (1 L volume [5]).

The predicted $c_L(t)$ aeration curves using the estimated K_La are plotted in figures 2-3 (continue lines), being in a satisfactory agreement with the experimental curves under variate aeration and mixing conditions. It should be pointed out that K_La estimated for higher aeration rates (more than 1.4 L min^{-1}) presents deviations from the increasing trend, due to a change in the small bubbles aeration regime, eventually affecting the mass transfer rate (results not presented here).

To correlate the calculated K_La with the operating conditions, various empirical relationships have been proposed in the literature, including the influence of a large number of factors: physical properties of the liquid phase, mixing conditions, gas solubility, temperature, sparger depth, aeration rate, vessel volume, baffles geometry, presence/absence of biomass flakes, etc. [1.5-7.15]). During the present experiment most of constructive/ operating parameters are kept constant (i.e. temperature, liquid volume, sparger depth and stirrer power). Consequently, a simplified correlation has been proposed, in the form:

$$K_La = b_o N_a^{b_1} U_g^{b_2} \ , \qquad (8)$$

where N_a= stirrer speeds, in rpm; U_g= gas superficial velocity, in m s^{-1}; $k=[b_o,b_1,b_2]$= empirical correlation coefficients. To simplify the estimation of k-vector, eq. (8) can be linearized in the form:

$$y = \ln(K_La) = \ln(b_o) + b_1 \ln(N_a) + b_2 \ln(U_g) . \qquad (9)$$

By using the transformed variables $y = \ln(K_La)$, $x_1 = \ln(N_a)$, $x_2 = \ln(U_g)$, the correlation model corresponds to the standard multi-variable linear form of: $y = \tilde{b}_o + \sum_{j=1}^{p} b_j x_j$ (p= number of independent variables). By applying the classical linear regression procedure with the least squares estimator [17] one obtains the estimate of the model parameters presented in table 1. Experimental error

Adequacy test		*Test value*	*Critical value*			*Conclusion*	
R (note a)		0.89	ca. 0.90			Ok	
s (note b)		0.35908	Avg. $y_{exp} = \bar{y} = 6.17$			Ok (ca. 5% of $\bar{y}$)	
χ^2 (note c)		15.593	$\chi^2(n-p;0.95) = 16.919$			adequate	
Parameter	*Estimate*	*95% confidence* (note d)	*t-test* (note e)	*Correlation matrix* (note f)		$\lambda_j / \tilde{\sigma}^2$ (note g)	*Conclusion*
$\ln(b_o)$	-9.9712	± 1.5713	14.355	1		1.86×10^2	Significant
b_1	1.8270	± 0.20112	20.550	-0.67	1	1.25×10^4	Significant
b_2	0.95531	± 0.16430	13.153	0.67	0.10 1	8.45×10^6	Significant

Table 1
MODEL ADEQUACY AND ESTIMATE QUALITY TESTS FOR THE CORRELATION OF EQ. (9)

Footnotes:

(a) R= multiple correlation coefficient, evaluated with the relationship:

$$\sum_{u=1}^{n}(y_i - \hat{y})^2 = (1-R^2)\sum_{u=1}^{n}(y_u - \bar{y})^2 \ ; \ \bar{y} = (\sum_{u=1}^{n} y_i)/n ;$$

(b) $s^2 = \left\| y_{exp} - \hat{y}_{model} \right\|_2^2 /(n-p)$ = model prediction variance; n=12 is number of experimental points; p=3 is the number of model parameters.

(c) Calculated $\chi_c^2 = \frac{s^2}{\sigma^2}$; $\chi^2(df;q)$ denotes the quantile of the χ^2-statistics with df degree of freedom and q confidence level;

(d) Confidence interval of the estimated parameter k_j, computed with the relationship:

$$k_j = \hat{k}_j \pm \{\sqrt{[V(\hat{\boldsymbol{k}})]_{jj}}\}\, t(n-p;97.5\%) \ ; \ V(\hat{\boldsymbol{k}})^{-1}_{[p,p]} = \sum_{u=1}^{n} \frac{1}{\sigma_u^2}\left[\frac{\partial y_u}{\partial \boldsymbol{k}}\right]^T_{[p,1]}\left[\frac{\partial y_u}{\partial \boldsymbol{k}}\right]_{[1,p]} ;$$

$\boldsymbol{V}$= estimate covariance matrix; $\boldsymbol{k} = [\ln(b_o), b_1, b_2]$;

$t(df;q)$= quantile of the Student-statistics with df degree of freedom and q confidence level;

(e) Student test for parameter significance:

$$t_j = \left| \frac{\hat{k}_j}{\sqrt{[V(\hat{\boldsymbol{k}})]_{jj}}} \right| > t(n-p;97.5\%) = 2.262.$$

(f) $\boldsymbol{R}$ is the parameter inter-correlation matrix calculated with the relationship:

$$\boldsymbol{R}(\hat{\boldsymbol{k}})_{[p,p]} = \{R_{ij}\}; \ R_{ij} = \frac{[V(\hat{\boldsymbol{k}})]_{ij}}{\sqrt{[V(\hat{\boldsymbol{k}})]_{ii}[V(\hat{\boldsymbol{k}})]_{jj}}} \in [-1,1] .$$

(g) The parameter 'ridge selection' test of Maria and Rippin [19]: $\lambda_j / \tilde{\sigma}^2 > 1-3$, where λ_j are eigenvalues of matrix $\boldsymbol{U}$:

$$\boldsymbol{U}(\hat{\boldsymbol{k}})_{[p,p]} = \sum_{u=1}^{n}\left[\frac{\partial y_u}{\partial \boldsymbol{k}}\right]^T_{[p,1]}\left[\frac{\partial y_u}{\partial \boldsymbol{k}}\right]_{[1,p]} + \tilde{\sigma}^2 \boldsymbol{I}; \ \tilde{\sigma}^2 = Min(\sigma_u^2) .$$

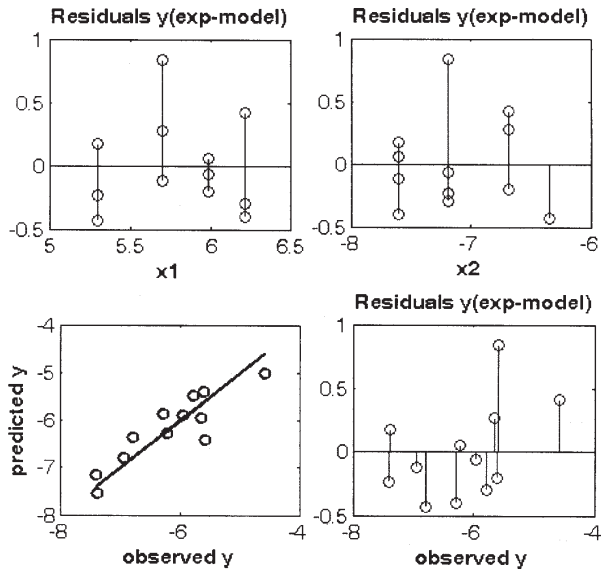
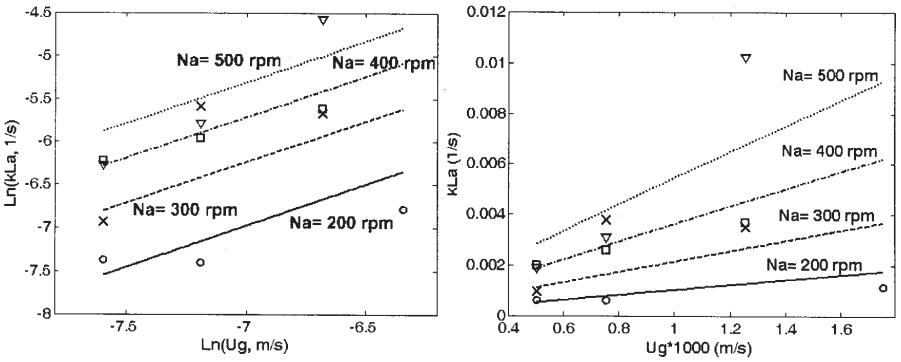


Fig. 4. Residual plots (O) for the empirical model of eq. (9).
(Up-left) Residuals [ln(K_La) (exp.) - ln(K_La) (model)] vs. $x1=ln(N_a)$.
(Up-right) Residuals [ln(K_La)(exp.) - ln(K_La) (model)] vs. $x2=ln(U_g)$.
(Down-left) Predicted $y=ln(K_La)$ vs. observed y.
(Down-right) Residuals [ln(K_La)(exp.) - ln(K_La) (model)] vs. observed y.



a) b)

Fig. 5. Experimentally evaluated K_La and predicted values by using the empirical logarithmic correlation of eq. (9) (a), or in the original variables eq (8) (b). Plotted curves correspond to N_a = 200 rpm (O,—), N_a = 300 rpm (X,- - -), N_a = 400 rpm (□,— . —.), and N_a = 500 rpm (∇, · · ·).

standard deviation (noise level) is of $\sigma_y \approx 0.1$; K_La unit is s^{-1}; N_a unit is rotations- per - minute (rpm); U_g unit is m s^{-1} ([17] for details on the estimate analysis methodology).

The estimate statistical analysis follows the standard methodology, including model adequacy and estimate significance tests (see the footnotes of table 1 [17-18]). Test results presented in table 1 indicate an adequate model (in the logarithmic form), i.e. a multiple correlation coefficient around 0.90, a standard deviation of model predictions around 5% of the observed value, and a satisfactory χ^2 adequacy test for 95% confidence level. The estimated parameters quality tests of table 1 indicate all model constants as being significant, that is small 95% confidence intervals, t-tests higher than the critical value (of 95% confidence), moderate correlation coefficients, and values of the ridge test ($\lambda_j / \tilde{\sigma}^2$) of [19] much higher than the critical threshold 1.

The residual plots presented in figure 4 confirm the satisfactory adequacy of the model. Indeed, the residuals are alternate positive and negative and of small values, while the predicted vs. observed y-plot indicate alternate values equally disposed in the vicinity of the graph diagonal. Such plots confirm the hypothesis of constant experimental noise (normally distributed), the linear character of the Log-correlation, and the absence of outliers in data [17-18]).

The plots of Log-model predictions as function of gas superficial velocity U_g in figure 5 (left) indicate a satisfactory agreement with the experimentally data. As expected, the residuals in absolute terms are however larger than those in logarithmic terms (fig. 5-right).

Conclusions

Precise evaluation of the mass transfer resistance in aerated bioreactors is of crucial importance for a correct interpretation of the enzymatic or bioprocess kinetic data, by separating the contribution of the gas-liquid transport to the oxygen consumption in bio-reactions. Instead of using the classical methodology of removing the dissolved oxygen by the addition of sodium sulphite, a non-invasive procedure is proposed by partially de-aeration of the bioreactor using nitrogen, followed by re-aeration, and measuring the dissolved oxygen concentration through time. Such a procedure is easily implementable on every laboratory-scale bioreactor, and has the advantage that the inert nitrogen does not change the characteristics of the liquid-phase. This preliminary step can be conducted before starting the study of the biological process.

The estimated K_La coefficient under various operating conditions can be then correlated with various factors, thus allowing to range the optimal aeration conditions for each system in a more systematic way. As expected, the mass transfer can be intensified by improving the mixing conditions and, under certain limits, the aeration rate. Other process parameters can also be used for such purposes.

Acknowledgment: Partial financial support of the European Commission through European Regional Development Fund and of the Romanian state budget, project POSCCE-02.1.2.-2009-2, ID 691, "New Mesoporous Aluminosilicate Materials for Controlled Relase of Biologically-Active Substances" is gratefully acknowledged.

References

1. MOSER, A., Bioprocess technology: Kinetics and reactors, Springer-Verlag, New York, 1988
2. DUNN, I.J., HEINZLE, E., INGHAM, J., PRENOZIL, J.E., Biological reaction engineering: Principles, applications and modelling with PC simulation, VCH, Weinheim, 1992
3.LIESE, A., SEELBACH, K., WANDREY, C. (Eds.), Industrial biotransformations, Wiley-VCH, Weinheim, 2006
4. BRENDA, The comprehensive enzyme information system, University of Köln, 2007. /www.brenda.uni-koeln.de/
5. NAJAFPOUR, G.D., Biochemical engineering and biotechnology, Elsevier, Amsterdam, 2007
6. JORGENSEN, S.E., Industrial waste water management, Elsevier, Amsterdam, 1979
7. TCHOBANOGLOUS, G., BURTON, F.L., Wastewater engineering: Treatment, disposal, and reuse, McGraw-Hill, New York, 1991
8. GOURICH, B., VIAL, C., EL AZHER, N., BELHAJ SOULAMI, M., ZIYAD, M., Chemical Engineering Science, **61**, nr. 18, 2006, p. 6218
9. TRAMBOUZE, P., Van LANDEGHEM, H., WAUQUIER, J.P., Chemical reactors: Design, engineering, operation, Edition Technip, Paris, 1988.
10. DECKWER, W.D., BECKER, F.U., LEDAKOWICZ, S., WAGNER-DOEBLER, I., Environmental Science and Technology, **38**, nr. 6, 2004, p.1858
11. WIKIPEDIA, The free encyclopedia, 2010. http://en.wikipedia.org/wiki/Henry%27s_law.
12.TREITZ, G., MARIA, G., GIFFHORN, F., HEINZLE, E., Jl. Biotechnology, **85**, nr. 3, 2001, p. 271
13. MARIA, G., Rev. Chim. (Bucharest), **56**, no. 11, 2005, p. 1078
14. MARIA, G., Computers & Chemical Engineering, **31**, nr.10, 2007, p. 1231
15. GALACTION, A.I., CASCAVAL, D., ONISCU, C., TURNEA, M., Chemical and Biochemical Engineering Quarterly, **19**, nr. 1, 2005, p. 87
16. GOMES, N., AGUEDO, M., TEIXEIRA, J., BELO, I., Biochemical Engineering Journal, **35**, nr. 3, 2007, p. 380
17. MARIA, G., Statistical data analysis and correlations. Distributions and estimators, Printech Publ., Bucharest, 2008 (in Romanian).
18. MARIA, G., Chemical and Biochemical Engineering Quarterly, **18**, nr. 3, 2004, p. 195
19. MARIA, G., RIPPIN, D.W.T., Chemical Engineering Science, **48**, nr. 22, 1993, p. 3855
20. STOENICA, M., LAVRIC, V., Time Scale Behaviour of a Biological Wastewater Treatment Facility, Rev. Chim. (Bucharest), **61**, no. 4, 2010, p. 407

Manuscript received: 9.06.2010