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Research Article

MASS TRANSFER COEFFICIENT EVALUATION FOR PILOT SCALE FERMENTER USING SODIUM SULPHITE OXIDATION METHOD

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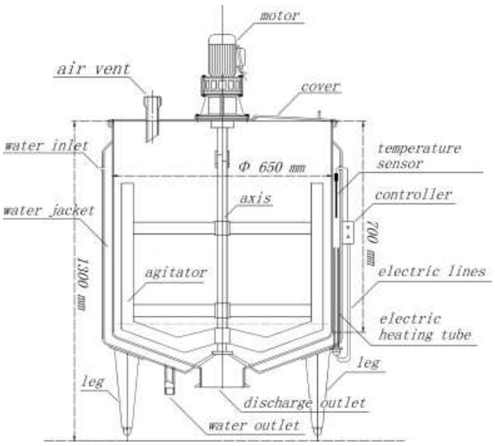
Abstract: An adequate supply of oxygen in aqueous solution becomes the focal point of interest when it comes to the growth and maintenance of most aerobic microbial and tissue cultures used for biochemical and pharmaceutical production. Unfortunately, oxygen mass transfer to the growth medium serves as a major growth limiting factor owing to it's low solubility in aqueous solutions. (Approximately 10 ppm at ambient temperature and pressure). The reaction rate is such that as oxygen enters the liquid phase, it is immediately consumed to oxidize the sulfite so that the rate of oxidation equals that of the oxygen transfer. Oxygen must at first be transferred from gas bulk through a series of steps onto the surfaces of cells before it can be utilized. Therefore the enhancement of gas-liquid mass transfer during aerobic cultures and fermentations is always put into priority. The present study involves using the 'Central Composite Design', a statistical technique to determine the parametric conditions for the optimum volumetric mass transfer coefficient in a pilot scale (40L) fermenter. The optimum volumetric mass transfer coefficient was found to lie outside the range of parameters studied and analytical expressions was obtained to predict the volumetric mass transfer coefficients for the parameter ranges studied using response surface methodology. The analytical expression was addressed to be significantly valid based on ANOVA results.

Keywords*:* Aqueous Solution, Aerobic Culture, Gas-Liquid Mass Transfer Coefficient, Pilot Scale Fermenter, Response Surface Methodology, ANOVA.

INTRODUCTION

A fermentation pilot plant is a small fermentation processing system which is operated to generate information about the behavior of the fermentation system to be used in design of larger industrial facilities. In fact fermentation pilot plant 'mimic' the industrial scale fermentation process by carrying out the industrial process on a smaller scale. Thus mimicking the industrial process and size is the main characteristics of pilot plant. However, the only difference pilot plant fermentation is still considered a research stage and not the final industrial production.



In aerobic bioprocesses the phenomenon of oxygen transfer plays a very crucial role and hence it's shortage would drastically affect the performance of the process concerned. Generally aqueous media is used for bioprocess practices where solubility of oxygen is very low owing to the presence of ionic salts and nutrients. At the same time the rate of oxygen utilization by the microorganisms is rather high. The amount of Dissolved Oxygen into the broths is limited by its solubility and mass transfer rate, as well as by its consumption rate on cells metabolic pathways (Calik et al. 1997). The enhancement in the rate of oxygen transfer is usually accomplished by agitation and is also for mixing nutrients and to keep the system homogeneous. However, there are limits to the speed of agitation, due to high power consumption (which is proportional to the cube of the speed of the electric motor) and the damage caused to the organisms due to excessively high tip speed. This is the reason why gas-liquid mass transfer is commonly the rate-limiting step in industrial-scale biochemical reactions. Thus, bioreactor design and scale-up focuses on providing optimum gas mass transfer. [1]

The oxygen mass transfer can be explained and

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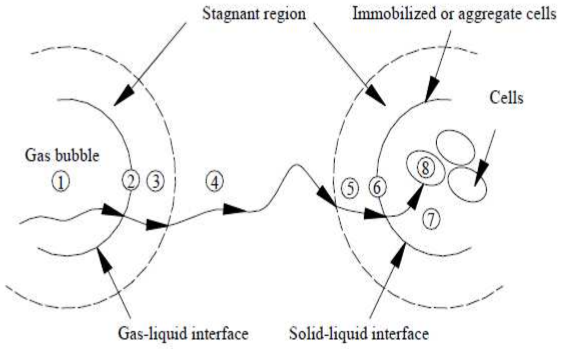
analyzed on the basis of volumetric mass transfer coefficient k_La (proportionality constant), taken as proportional to the concentration gradient. The maximum value of the concentration gradient is limited owing to the low solubility of most gases associated to aerobic fermentation, notably oxygen. Therefore, the maximum mass transfer rate from the gas to the liquid in the bioreactor can be estimated by the product $k_La.C^*$, C^* being the saturation concentration in the liquid phase. Thus the coefficient (k_La) plays an important role in design, scale-up and economy of the process. [2]

To eliminate dissolved oxygen limitations and allow cell metabolism to function at its optimum, the dissolved oxygen concentration at every point in the fermenter must be above critical. Choice of substrate for the fermentation can also significantly affect oxygen demand. As far as aerobic fermentation is concerned oxygen molecules have to cross the hurdle of a series of transport resistances before the cells can utilize them. [1]



Steps for oxygen transport from gas bubble to cell

In order to illustrate the mass transfer of gases into liquid typically two main parts are to be dealt with: the micro model, describing the mass transfer between the gas and the liquid phase and the macro model describing the mixing pattern within the individual gas and liquid phases.

Frequently applied micro models include:

1. The stagnant film model (Whitman, 1923).
2. The Higbie penetration model (Higbie, 1935).
3. The Danckwerts surface renewal model (Danckwerts, 1951).
4. The film penetration model (Dobbins, 1956 and Toor & Marchello, 1958).

The stagnant film model assumes the presence of a stagnant liquid film, while the penetration model and the surface renewal model approach the gas-liquid mass transfer using dynamic absorption in small liquid elements at the contact surface. All micro models mentioned above assume the presence of a well-mixed liquid bulk. This may limit the application of these models to systems where a liquid bulk is present, for example absorption in a tray column or mass transfer in a stirred tank reactor.

Further enhancements in the oxygen transfer rate are, of course, also possible with the application of one or more of the following; increased back pressure, oxygen enriched air or a small separate sparged flow of oxygen.[2]

MATERIALS AND METHODS

Sodium Sulphite Oxidation Method:

A chemical model system is used as an artificial oxygen consumer. This method here is based on the reaction of sodium sulphite as a reducing agent, with the dissolved oxygen to produce sulphate, in the presence of a catalyst (usually a divalent cation such as Cu^{++} or Co^{++}). The reaction can be expressed as

$$2SO_4^{-2}-O_2-2SO_3^{-2}=0$$

There is a concentration range of sodium sulphite (from 0.04 to 1N) for which the reaction is so fast that oxygen concentration can be assumed to be zero. The reaction rate is much faster than the oxygen transfer rate; therefore, the rate of oxidation is controlled by the rate of mass transfer, and measuring the overall rate, the mass transport rate can be determined. The reaction rate is a complex function of the catalyst concentration and the operational conditions must be controlled in order to obtain reproducible measurements. The rate of sulphite consumption is determined and k_La is calculated from

$$-dC_{Na2SO3}/dt=2k_LaC^*$$

Where, dC_{Na2SO3}/dt- rate of consumption of sodium sulphite (mol/litresec)

Linek and Vacek in 1981 have reviewed the use of the sulphite oxidation method, as a model reaction of known kinetics and it's capacity for accurately determining mass transfer characteristics. The sodium sulphite oxidation method is relatively easy to carry out. This technique has been used in a large number of works. However, this method has the limitation that, because some of its physical properties are very different from those of fermentation broths, the hydrodynamics of the solution is different, mainly due to the influence of those properties on bubble size. [3,6]

Experimental design and data analysis: Central composite design (CCD):

The Central composite design was employed for the optimization of process conditions (Khuri and Cornell, 1987; Mark J. Kiemele and Stephen R, 1999). In the present work, statistical design approaches have been proposed to optimize the process conditions on the evaluation of mass transfer coefficient. A 2^2-factorial Central composite design was employed for the optimization of process conditions viz., Impeller speed and air flow rate. The objective of this section was to find optimal conditions of the process conditions in order to enhance the mass transfer coefficient.

Central composite designs are very efficient providing much information on experiment variable effects and overall experimental error, in a minimum number of required runs.

The effect of Impeller speed and air flow rate. On Gas–Liquid phase mass transfer coefficient after optimization were observed & compared. The effect of the process conditions was studied using a second order Central composite design (CCD) (Khuri and Cornell, 1987). The Chemical parameters studied such as temperature and initial pH kept constant for the determination of mass transfer coefficient. The variables i.e, Speed of impeller and air flow rate were taken as the independent variables.

According to the Central composite design, the total number of treatment combinations was $2^k + 2k + n_o$ where 'k' is the number of independent variables and n_o is the number of repetition of experiments at the centre point. The total number of design points is thus $N=2^k + 2k + n_o$.

The significant variables like Speed of impeller & Air flow rate were chosen as the critical variables and designated as X_1 and X_2 respectively. The low, middle, and high levels of each variable were designated as –, 0, and + respectively. -α and +α are the extreme levels in the range studied for each variable, -1 and +1 are intermediate levels between the central and extreme levels of each variable, and 0 is the central level in the range studied for each variable. The experimental range for Speed of impeller & Air flow rate are chosen for this study (obtained using Design Expert Software, Stat-Ease, U.S.A.) is given in Table

Table.1: Experimental range and levels of impeller speed and air flow rate. in Central composite design (CCD).

Variable	Parameter	Level				
		-α	-1	0	+1	+α
X_1	Speed of impeller	217.16	300	500	700	782.84
X_2	Air flow rate	4.76	6	9	12	13.24

A 2^2-factorial central-composite-experimental-design was employed and all in duplicate, leading to 13 sets of experiments, was used to optimize the mass transfer coefficient. Experimental plan was employed for the optimization of impeller speed and air flow rate.. For statistical calculations, the variable X_i were coded as x_i according to the following transformation

$$x_i = (X_i - X_o)/\delta X \qquad (1.1)$$

Where:

x_i = dimensionless coded value of an independent variable X_i,

X_i = actual value of an independent variable,

X_o = actual value of an independent variable X_i at the center point, and δX = step change.

The variables are preferably used in coded form for two reasons:

1. Computational ease and increased accuracy in estimating the model coefficients.
2. Enhanced interpretation of the coefficient estimates in the model.

The specific codes are:

Coded value of the impeller speed, $x_1 = [X_1 - 500] / 300$.

Coded value of the air flow rate, $x_2 = [X_2 - 9] / 6$.

Where $X_1$and X2 are the actual values of the independent variables respectively, where x_1, x_2 are the coded values of the independent variables viz., speed of impeller, air flow rate respectively. The values in the parenthesis are corresponding to decoded (actual) values.

The optimum mass transfer coefficient is taken as the dependent variable or response Ŷ. Regression analysis was performed on the data obtained. The behaviour of the system was explained by the following second order polynomial Equation 3.3 (Khuri and Cornell, 1987).

$$\hat{Y} = \beta_o + \Sigma\beta_i x_i + \Sigma\beta_{ii} x_i^2 + \Sigma\beta_{ij} x_i x_j \qquad (1.2)$$

Where

Ŷ = predicted response

β_o = offset term,

β_i = linear effect,

β_{ii} = squared effect, and

β_{ij} = interaction effect.

x_i and x_j = coded value of independent variables.

The regression equation was optimized for maximum value to obtain the optimum conditions using MATLAB version 7.0. The second order polynomial equation was obtained using Design-Expert software [5].

RESULTS AND DISCUSSIONS

The volumetric mass transfer coefficient was determined using sodium sulphite oxidation method. The experiments were carried out in 40 L (working volume) fermenter. The conventional practice of single factor optimization by keeping other involving factors at unspecified constant levels does not depict the combined effect of all the factors involved. Also this method requires carrying out a number of experiments to determine the optimum levels, which will not give true values. Optimizing all the affecting parameters combined by statistical experimental design can eliminate these drawbacks of single factor optimization process. The effect of the process conditions namely impeller speed and air flow rate. Where studied using a second order central composite experimental design (CCD). A total of 13 experiments with different combinations of impeller speed and air flow rate and performed using central composite design to find the parameter conditions where the optimum volumetric mass transfer coefficient occurs. Table.2 show the comparison between experimental and predicted values for the volumetric mass transfer coefficient using sodium sulphite oxidation method. The error was well within + 10 % indicating that the empirical expression for the prediction of volumetric coefficient is valid. The expression obtained in term of coded factors is given by the equation, $Y_1 = 294.83 + 0.75x_1 + 28.62x_2 - 16.90\ x_1x_2 + 14.59\ x_1^2 - 10.51\ x_2^2$ where Y_1 is the response variable i.e., volumetric mass transfer coefficient, x_1 and x_2 are coded values of independent variables, i.e., impeller speed and air flow rate, respectively. Actual form of the empirical expression gives the predicted value of volumetric mass transfer coefficient. $Y_2 = 76.9739 - 0.010758X_1 + 44.64158X_2 - 0.02817\ X_1\ X_2 + 0.00036X_1^2 - 1.1679X_2^2$ whereY_2 is the response variable, Volumetric mass transfer coefficient. X_1 and X_2 actual values of independent variables, i.e., impeller speed and air flow rate, respectively.

Table.2: Comparison of experimental and predicted values of volumetric mass transfer coefficient for 40 L sodium sulphite oxidation method

Run	Impeller speed (rpm)	Air flow rate (lpm)	Volumetric mass transfer coefficient (hr^{-1})		Error- (%)
			Experimental	Model	
1	500	9	296.205	294.998	-4.073
2	782.84	9	325.825	324.802	0.313
3	217.16	9	330.547	330.547	-2.29
4	500	9	292.77	294.998	0.761
5	500	9	292.77	294.998	0.761
6	500	9	296.205	294.998	-0.838
7	300	12	349.436	349.436	1.647
8	500	13.24	301.034	301.034	4.381
9	700	12	321.962	321.962	-3.347
10	500	4.76	254.994	254.994	-8.493
11	700	6	273.882	287.756	5.065
12	500	9	296.205	294.998	-0.407
13	300	6	233.744	252.644	8.085

The independent and the dependent variables were fitted to the second-order model equation. They were examined in terms of the goodness of fit. The goodness of fit of the regression equation Y1 was evaluated by the coefficient of determination (R2) and the coefficient of relation (R). The coefficient of determination (R2) is a measure of total variation of observed values of extracted oil about the mean explained by the fitted model. The coefficient of correlation (R) explains the correlation between the experimental and predicted values from the model. A good model equation explains most of the variations in the response. The coefficient of determination (R^2) is 0.8783. This value indicates that the response model can explain 87.83% of the total variability in the responses. The coefficient of correlation (R) is 0.9371.The closer value of coefficient of correlation (R) to unity is the better. Statistical testing of the model was done in the form of variance (ANOVA), which is required to test the significance and adequacy of the model. The reliability of the suggested model was tested using the Fisher's statistical test (F). The results of statistical testing using ANOVAs are given in Table 3.

Values of " Probability (P) > F"less than 0.05 indicate that the model terms are significant. The ANOVA of the regression model corresponding to quadratic for volumetric mass transfer coefficient Table.3 demonstrates that the model is highly significant, as it is evident from the calculated F-value (= 10.10) and a very low probability value (Probability (P) > F = 0.0009). Moreover the computed F-value (F= 10.10) is much greater than the F value ($F_{0.0009(5,7)}$= 9.52) obtained from the standard distribution table, so the null hypothesis is rejected at 5% α level of significance. From Figure.1 it can be observed that a stationary point exists although it is outside the range based on the shape of the contour plot. The response surface plot shown in Figure.2 for the chosen model Y_1illustrates the three dimensional relationship for the effects of impeller speed and air flow rate. on volumetric mass transfer coefficient. The response surface indicates that the volumetric mass transfer coefficient increases with decrease in impeller speed and subsequent increase in air flow rate. This result indicates that two variables had mutually dependent influence on the volumetric mass transfer coefficient.

Table.3: Analysis of Variance (ANOVA)

Source	Sum of squares	Degrees of freedom	Mean square	F value	*Probability(P)>F
Model	10258.27	5	2051.65	10.10	0.0009 significant
Error	1421.78	7	203.11		
Total	11680.05	12			

F value F0.0009 $_{(\cdot 5,7)}$ = 9.52 obtained from the standard distribution table. *Values of " Probability (P) > F"less than 0.05 indicate that the model terms are significant.

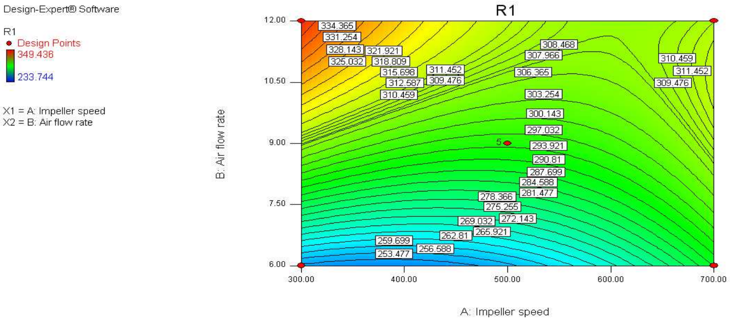


Figure.1: Isoresponse contour plots showing the effect of impeller speed and air flow rate and their interactive effect on the volumetric mass transfer coefficient for 40 L sodium sulphite oxidation method

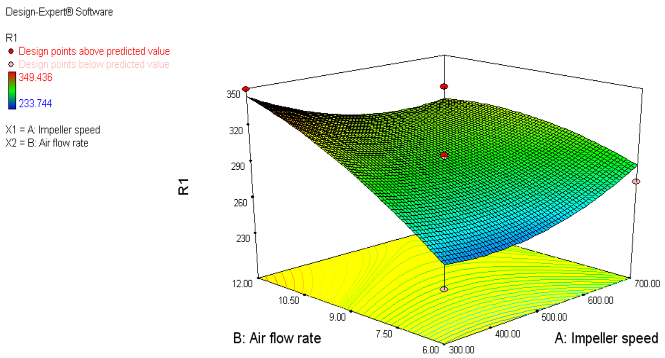


Figure.2: Response surface plot showing the effect of impeller speed and air flow rate and their interactive effect on the volumetric mass transfer coefficient for 40L sodium sulphite oxidation method .

CONCLUSION

Evaluation of mass transfer coefficients in fermenters were studied using central composite design to get the optimum value. A total of 13 experiments for each set were employed to determine the volumetric mass transfer coefficients. The order of the reaction with respect to oxygen consumption for 40L sodium sulphite oxidation method was found to be first order and zero order for the case of sodium sulphite oxidation. Optimum volumetric mass transfer coefficient was found from response surface methodology to be outside the range of parameters studied. Analytical expressions for predicting the volumetric mass transfer coefficient for the range of impeller speed and air flow rate. tested were obtained using response surface methodology.

Nomenclature:

k_La = Volumetric mass transfer coefficient, C* = Equilibrium concentration in moles /litre, t = Time in minutes or sec and C_{Na2So3} = Concentration of sodium sulphite in mol/litre.

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