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MODELLING OF MASS TRANSFER COEFFICIENT OF CO_2 IN STIRRED TANK BIO-REACTORS USING RESPONSE SURFACE METHODOLOGY (RSM)

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Abstract

In this study, the influence of three operational parameters (agitation rate, gas flowrate and gas hold up) and their interactions on the overall volumetric mass transfer coefficient (k_La) of carbon dioxide (CO_2) for microalgae cultivation in stirred tank bioreactors were investigated using Response Surface Methodology (RSM). The three variables were modelled with overall mass transfer coefficient as the response using historical data design in the Design Expert 6.0.8. The response of the model developed was in line with data used for model development from the R^2 values of the developed model. The study revealed that the historical data RSM design is an efficient statistical technique for providing appropriate empirical model for relating the operational parameters and predicting the optimum operating conditions affecting the k_La of CO_2 which is the major determinant of the amount of the same gas consumed by microalgae to achieve maximum growth in bioreactors.

***Keywords**: Overall volumetric mass transfer coefficient; Response Surface Methodology (RSM); Historical data design; Empirical model; Optimization.*

1. Introduction

Microalgae and macroalgae have numerous unique attributes which makes them useful in various applications ranging from biodiesel production to wastewater treatment. Due to their abundant oil content, microalgae have been regarded as the primary raw material capable of yielding enormous quantities of biodiesel that could meet global demands, while in wastewater treatment plants, the contaminants serve as substrates for algae growth in which way the water is being purified [1]. In stabilizing our climate, reduction of CO_2 emissions from bioreactors have also been made possible using microalgae as substrate for sustenance [2]. In all these applications, microalgae are more commonly utilized because of their extraordinary potential for cultivation as energy feedstock and rapid growth rate due to their unicellular nature.

Traditionally, microalgae are cultivated in open systems and some of the shortcomings of these methods are contamination problems, inability to control process variables and determination of optimum conditions that promotes growth of microalgae. The identified shortcomings were addressed in cultivation of algae in closed system [3] where a stirred tank bioreactor was used as the closed systems used for cultivation of microalgae [3]. Bioreactors aid in high energy dissipation (heat and mass transfer rate) and offers excellent mixing based on parameters that can be regulated in the reactor. Some of the parameters that affect the mass transfer in microalgae are stirrer's speed, composition of gas used and gas flow rate [4].

The efficient transfer of CO_2 from gaseous to liquid phase is a pertinent parameter involved in the design, operation and scale-up of bioreactors [5]. The importance of this parameter is evident in the usefulness of CO_2 to microalgae in only the dissolved form, the carbon constituent being an essential nutrient for the cultivation of microalgae [6]. Hence the need to design a stirred tank bioreactor which operates at conditions that factor in maximum CO_2 transfers from the gaseous to liquid phase.

It is the general practice of industries involved in the production of microalgae from CO_2 to base their estimations of the CO_2 consumption by the living cells on optical density, pH and dissolved oxygen (DO). However, a more accurate parameter for measuring the transfer rate of CO_2 from gaseous to liquid phase is the overall volumetric mass transfer coefficient as opposed to biomass quantification, pH monitoring and DO measurements which only provide approximate information on CO_2 utilization by microalgae [7]. The overall volumetric mass transfer coefficient, apart from being important in estimating reactor performance, also gives the optimum quantity of CO_2 required by microalgae to achieve maximum growth [8] as well as the actual amount of the gas transferred. Thus, the measure of the amount of CO_2 consumed by microalgae compared to the amount available is better estimated by the overall volumetric mass transfer coefficient [4].

The overall k_La is defined as the product of mass transfer coefficient and interfacial area. The mass transfer coefficient should not be confused with k_La as the former refers to the ratio of flux to concentration difference [9]. k_La is determined through experiments or empirical correlations to measure the mass transfer of gas into liquid to eliminate the difficulties encountered with other phenomena in determining interfacial area [5]. Among the factors affecting k_La of CO_2 in a microalgal solution as reported in literature are pH of culture media, superficial gas velocity, surface tension, viscosity, gas hold up, stirrer agitation rate and geometrical parameters of the bioreactor (type, distributor or stirrer design) [5,10-14].

A plethora of mechanistic models and correlations for determining k_La have been developed [9,15-16] and efforts have recently been made for theoretical prediction of the same. However, bulk of these researches have been developed for bubble columns and airlifts with only a handful dealing with k_La determination in stirred tanks bioreactors [11,13,17-19]. The other models developed in this field are that of Royce and Thomhill [20] & Doucha *et al.* [16] where mass transfer coefficients of CO_2 and Oxygen (O_2) in fermentation broth were developed while that of Babcock *et al.* [15] which estimated the overall mass transfer coefficient of CO_2 ($(K_La)_{CO_2}$) in three different media: tap water, sea water and algal culture in a horizontal tubular reactor. A major bottleneck to this approach is that the overall mass transfer coefficient of O_2 ($(K_La)_{O_2}$) should be determined experimentally first before $(K_La)_{CO_2}$ can be evaluated which means that $(K_La)_{CO_2}$ are mostly approximated values.

Response Surface Methodology (RSM) is a widely used tool for predicting the relationship between response and process variables because it is effective in optimizing the response function and predicting future responses after it has developed an empirical regression model statistically from appropriate experimental data [21-22]. It provides information on the effect of factor interactions on the behaviour of a response [23]. From the several design types available in RSM: Box–Behnken, central-composite, one-factor, optimal and historical-data, the historical data is the most preferred choice for this study as it can accommodate all available data into a blank design layout from an already conducted experiment [24]. Promising results were recorded when historical data design tool box in the Design Expert software was used for modelling of interactions between or among variables when design of experiment approach was not used to design before the start of experiment. Some of the work that was reported here are Salam *et al.,* [25], Salam *et al.,* [26] & Aremu *et al.,* [27] where historical design in the Design Expert software was used for model development and captured the interactions among variables.

It has been established by the preliminary study conducted by Kazim [28] that agitation rate, gas flowrate and gas hold up, are significant factors influencing k_La which is used to estimate the amount of CO_2 transferred from gaseous to liquid phase. This paper is aimed at developing an empirical model which would explain the effect of the interactions of the aforementioned parameters on the k_La of CO_2 for microalgae cultivation in stirred tank bioreactors, thereby eliminating the need for experimental determination of secondary parameters or derivatives. The optimum conditions will also be evaluated from the optimization of the response.

2. Methodology

The steps used in the description of data used, the experimental design used for the regression analysis, analysis of variance, model validation and numerical optimization of the variables used in the model development are described in this section below:

2.1. Experimental design and model regression

The historical data design in the Design Expert software version 6.0.8 (Stat-Ease Inc., Minneapolis, USA) was used for this study. Historical data experimental design, with categorical factor of 0, was employed in modelling and optimizing the k_La of CO_2 for microalgae cultivation in stirred tank bioreactors. The experimental result of Kazim [28] was used for the study. This publication has three operational parameters agitation rate, gas flowrate and gas hold up respectively. The parameters were operated within two ranges (minimum (-1) and maximum (+1)). The minimum and maximum levels of these three variables were: 150 and 400 rpm for agitation rate; 1100 and 3500 mL/min for gas flowrate and 0.027 and 0.070 for gas hold up respectively. A total of 16 experimental runs at different variations of the three parameters identified from Kazim [28] were used for modelling and optimizing purpose.

The general empirical model equation used for the modelling can be represented with the aid of the second-order quadratic model as shown in Equation 1. The empirical model can be used to study and analyse the interaction between or among [29-31].

$$Y = b_o + \sum b_i X_i + \sum b_{ii} X_i^2 + \sum b_{ij} X_i X_j + e_i \quad (1)$$

where Y is the predicted response; n is the number of factors; X_i and X_j are the coded variables; b_o is the constant coefficient; b_i, b_{ii}, and b_{ij} are the primary parameter, quadratic, and interaction coefficients, respectively; i and j are the index numbers for factors; and e_i is the residual error. The operational parameters, their designated symbols, response and range of conditions are summarized in Table 1.

Table 1. The variables and their range of values

Operating Parameters	Symbols	Ranges	Low Coded	High Coded
Agitation Rate (rpm)	A	150 – 400	-1	+1
Gas Flowrate (mL/min)	B	1100 - 3500	-1	+1
Gas Hold Up (-)	C	0.027 – 0.070	-1	+1
Response	Symbol	Analysis	Minimum	Maximum
Volumetric Mass Transfer Coefficient (h^{-1})	Y_1	Polynomial (Quadratic)	3.00	14.16

The validity of the developed model will be express by the coefficient of determination (R^2) and coefficient of adjusted determination (Adj-R^2) while the statistical significance will be verified with the F-test and the adequate precision ratio.

2.2. Optimization of operational variables and response

Numerical optimization tool in the Design Expert software will be used to determine the values of each of the three variables used for model development required for maximization of volumetric mass transfer of CO_2 into microalgae. The following steps were taken prior to the optimization in order to identify the criteria of the numerical optimization: the goal factors for agitation rate, gas flowrate and gas hold up were set to "is in range" while that of the response, k_La was set to "maximum". The upper limit of the response was the maximum response obtained from the interactions of the parameters considered.

3. Results and discussion

The results obtained from the modelling of volumetric mass transfer of CO_2 into microalgae were presented where model fitting, analysis of variance and model validation were considered. Other areas presented in this work are interactions of the variables with each other and their surface interactions.

3.1. One factor and interaction behaviour of the variables

The individual effects of each of the operational parameters, i.e. agitation rate, gas flowrate and gas hold up on the $(K_L a)_{CO_2}$ in stirred tank bioreactors are presented in Figure 1 (i-iv). Figure 1 (i) depicted the effect of agitation rate on the $(K_L a)_{CO_2}$ at constant gas flowrate and gas hold up. A direct relationship between $k_L a$ and agitation rate was observed with a slight increase in $k_L a$ from 7.1205 to 9.5943 h^{-1} as agitation rate increased from 150 to 400 rpm. Figure 1 (ii) illustrated the effect of gas flowrate on the $(K_L a)_{CO_2}$ at constant agitation rate and gas hold up.

The response decreased remarkably from 14.1958 to 2.5189 h^{-1} as the gas flowrate increased from 1100 to 3500 mL/min thereby indicating an inverse relationship between $k_L a$ and gas flowrate. Figure 1 (iii) showed the effect of gas hold up on $(K_L a)_{CO_2}$ at constant agitation rate and gas flowrate. There was a significant increase in the $k_L a$ values from -26.1252 to about 9 h^{-1} following increase in gas hold up from 0.03 to 0.53. However, the response decreased to -6.6213 h^{-1} with decrease in gas hold up to 0.07. Thus, agitation rate has a direct effect while gas flow rate has an indirect effect on the $(K_L a)_{CO_2}$ values in stirred tank bioreactors. Gas hold up however, has both direct and indirect relationship on the same response.

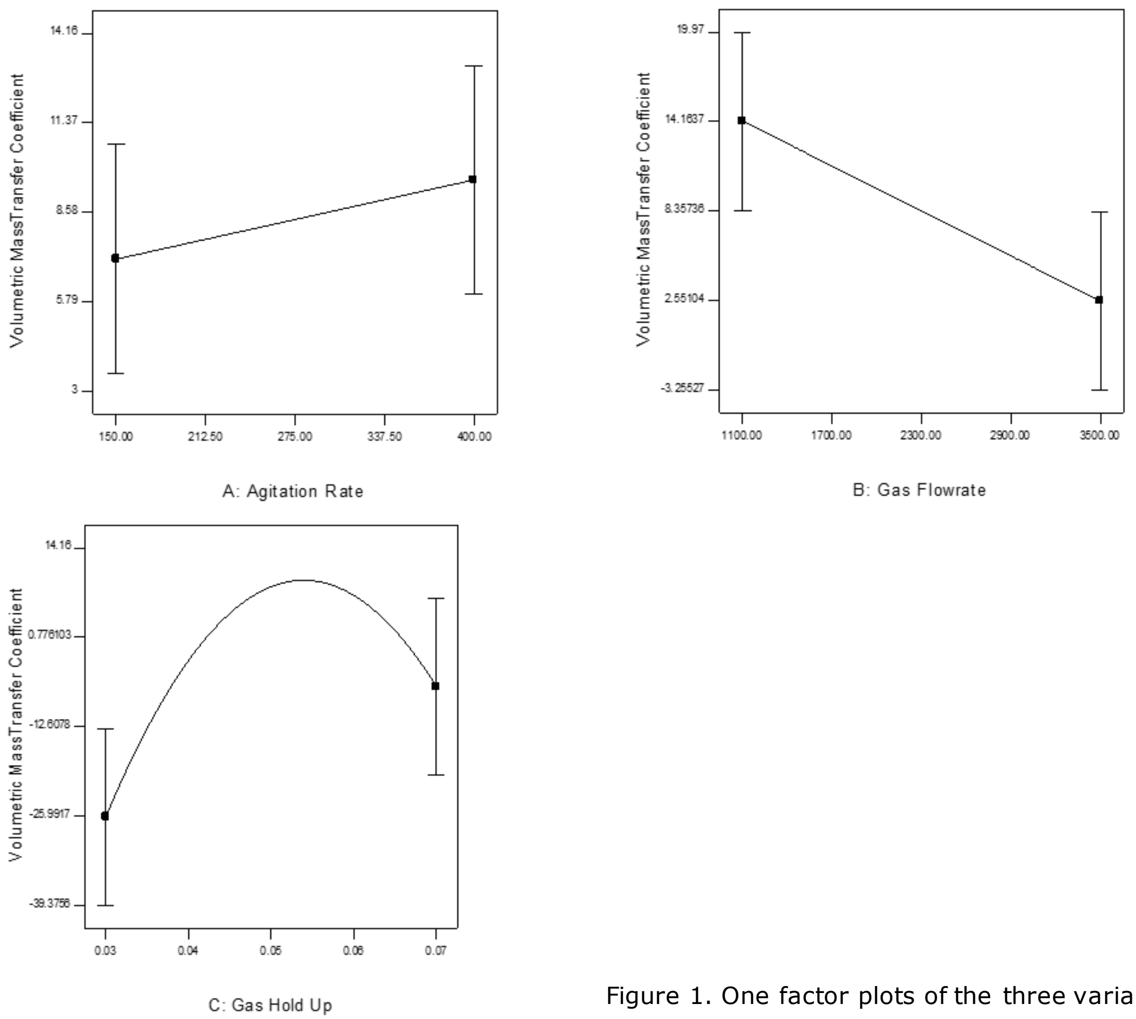

Figure 1. One factor plots of the three variables

3.2. D Response surface plots of the model

Three dimensional (3D) plots were analysed in this work to investigate the interactive effects of the three variables used in this study on the $(K_L a)_{CO_2}$ in the stirred tank bioreactors.

In these plots, the interactive effects of two operating parameters were considered while the other parameter was kept constant. They are shown in Figure 2 (i – iii).

The synergistic effect of agitation rate and gas flowrate on $(K_L a)_{CO_2}$ in the stirred tank bioreactors at constant gas hold up is depicted by Figure 2(i). At high gas flowrate of 3500 ml/min, an increase in the agitation rate of the stirred tank bioreactor from 150 to 400 rpm yielded an appreciable reduction in the $(K_L a)_{CO_2}$ in the bioreactor from 4.31 to 0.73 h^{-1}. However, when the gas flowrate was 1100 mL/min, the $(K_L a)_{CO_2}$ increased substantially from 9.93 to 18.46 h^{-1} with the same increase in agitation rate. The increment of k_La was more pronounced at high agitation rate following reduction in the gas flow rate than it was at low agitation rate.

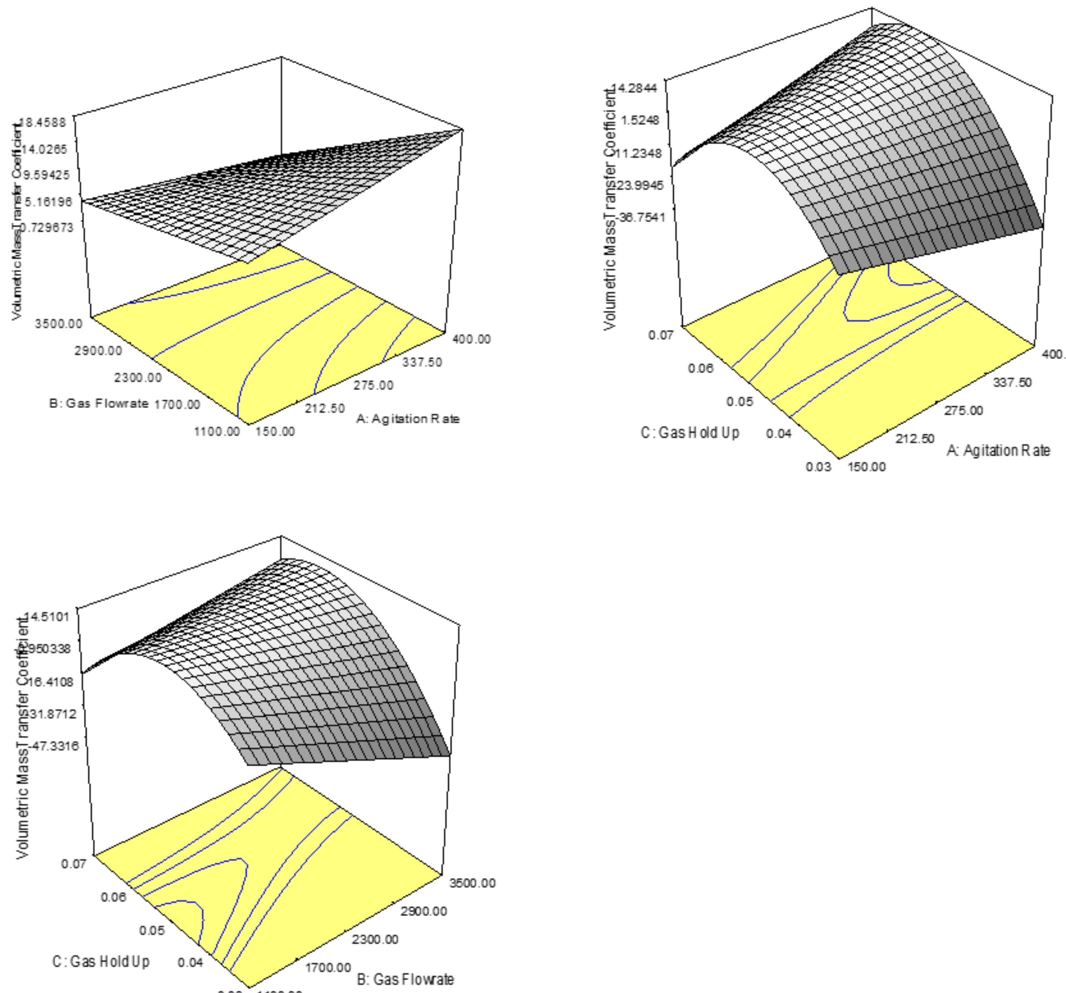

Figure 2. 3D plots of the interactions between the variables

Figure 2 (ii) show the interactive effect of agitation rate and gas hold up on the $(K_L a)_{CO_2}$ in stirred tank bioreactors when the gas flowrate was constant. An increase in the $(K_L a)_{CO_2}$ from -19.72 to 6.48 h^{-1} was obtained at high gas hold up of 0.07 with increase in agitation rate of the stirred tank bioreactor from 150 to 400 rpm. Meanwhile, k_La reduced from -15.50 to -36.75 h^{-1} at considerably low gas hold up of 0.03 with equivalent increase in agitation rate. The k_La of CO_2 was found to reduce at low agitation rate with increase in gas hold up in contrast to high agitation rate of the bioreactor where there was a large increase in k_La.

The effect of interaction between gas flowrate and gas hold up on the $(K_L a)_{CO_2}$ in the stirred tank bioreactor was illustrated in Figure 2 (iii). This interaction effect was considered at a constant agitation rate of the bioreactor. When the gas flowrate was increased from 1100 to 3500 mL/min at a gas hold up of 0.07, the $(K_L a)_{CO_2}$ in the stirred tank bioreactor increased from -16.15 to 2.91 h^{-1}. At gas hold up of 0.03, however, the same increase in the gas flowrate generated a relatively large decline in the k_La from -4.92 to -47.33 h^{-1}. In similar fashion to the observation in Figure 2 (ii), $(K_L a)_{CO_2}$ was found to reduce at low agitation rate with increase in gas hold up while at high agitation rate of the bioreactor, k_La values substantially increased.

These plots indicated that gas hold up is the most significant operational parameter influencing the $(K_L a)_{CO_2}$ in the stirred tank bioreactors as it was observed in the results reported by Kazim (2012). This is clearly expressed by Figure 2 (ii) and (iii) where k_La decreased significantly at low gas hold up and increased remarkably at high gas hold up irrespective of the other parameters involved.

3.3. Model fitting

The developed empirical model for the prediction of $(K_L a)_{CO_2}$ and contribution of each of the model parameters were analysed in Table 2. The prediction of the $(K_L a)_{CO_2}$ using historical design was generated using Equation 2 and shown in Table 2**.**

Table 2. Comparison of the experimental data used and model prediction.

Std	Run	Experimental variables			Response	
		Agitation rate (rpm)	Gas flow rate (mL/min)	Gas hold Up	Actual k_La (h^{-1})	Predicted k_La (h^{-1})
12	1	150	1100	0.027	3.00	2.68
16	2	200	1100	0.03	4.44	4.29
5	3	300	1100	0.035	7.20	7.33
6	4	400	1100	0.039	10.80	10.87
1	5	150	2000	0.037	3.36	3.93
14	6	200	2000	0.04	4.92	5.23
2	7	300	2000	0.047	9.48	9.52
4	8	400	2000	0.052	14.16	14.05
11	9	150	2900	0.044	3.72	3.47
8	10	200	2900	0.049	6.12	5.83
10	11	300	2900	0.058	9.48	9.31
7	12	400	2900	0.064	13.68	13.43
9	13	150	3500	0.049	4.32	4.60
13	14	200	3500	0.053	6.60	6.28
15	15	300	3500	0.062	9.48	9.67
3	16	400	3500	0.07	12.72	12.98

The polynomial regression analysis for the prediction of the $(K_L a)_{CO_2}$ shown in Equation 2 was developed through manual reduction of larger insignificant terms. The actual terms in the Equation 2 are three-individual model terms, one-quadratic term and three-two-parameter interaction terms respectively.

$$k_L a = -18.44626 - 0.15784 * Agitation\ Rate - 0.028207 * Gas\ Flow\ Rate + 3058.95308 * Gas\ Hold\ Up - 53500.56326 * Gas\ Hold\ Up2 - 0.0000201743 * Agitation\ Rate * Gas\ Flow\ Rate + 4.41517 * Agitation\ Rate * Gas\ Hold\ Up + 0.59566 * Gas\ Flow\ Rate * Gas\ Hold\ Up \quad (2)$$

The empirical model includes all the factors in consideration, thereby eliminating the need for experimental determination of theoretical parameters required by mechanistic models.

3.4. Analysis of variance (ANOVA) and statistical significance of the model

For the optimization of $(K_L a)_{CO_2}$ in the stirred tank bioreactor, analysis of variance (ANOVA) values were obtained for the quadratic regression model in Equation 2. The ANOVA results derived from the historical data utilized for this study are listed in Table 3. The p (or prob) values depicted the significance of each of the coefficients as well the interaction effectiveness between each independent variable. The p-value < 0.0001 and the model F-value of 215.54

(a large value occurring due to noise) for the second-order equation, suggested that the regression model is statistically significant. The significance of the regression coefficients is also depicted in Table 3. P-values < 0.05 indicated that the model terms are significant at 95% confidence level.

Table 3. ANOVA for response surface reduced quadratic model

Source	Sum of squares	Degree of freedom	Mean square	F-Value	Prob > F	Remark
Model	211.19	7	30.17	215.54	< 0.0001	significant
A	0.045	1	0.045	0.32	0.5851	
B	0.38	1	0.38	2.73	0.1370	
C	0.42	1	0.42	2.98	0.1224	
C^2	2.57	1	2.57	18.39	0.0027	
AB	0.53	1	0.53	3.80	0.0872	
AC	2.41	1	2.41	17.19	0.0032	
BC	1.88	1	1.88	13.43	0.0064	
Residual	1.12	8	0.14			
Cor Total	212.31	15				

From the ANOVA, it can be observed that three (3) of the four (4) model terms (C^2, AC and BC) are significant (not considering those required to support hierarchy: A, B, C). As earlier stated, model reduction by manual exclusion of few insignificant terms, was done to improve the predictive performance of the model [24,32]. The significant model terms have synergistic effect on the regression model while insignificant terms have antagonistic effect. Therefore, model factors C^2, AC and BC positively contribute to the model while A, B, C and AB have negative impact on the developed model. The most influential model parameter was C^2 because it had the lowest p-value.

3.5. Model validation

Since adequate precision measures the signal to noise ratio and a ratio value greater than 4 is desirable, the quadratic model of $(K_L a)_{CO_2}$ for microalgae cultivation with adequate precision ratio of 42.96 show an indication of an adequate signal. The quadratic regression model fitting was analysed by the coefficient of determination, R^2 which gave a high value of 0.9947 for the $(K_L a)_{CO_2}$ from the ANOVA results. A reasonable agreement of the R^2 with the Adj-R^2, is of great importance. The value of Adj-R^2 obtained was 0.9901. Therefore, the proximity of the R^2 and Adj-R^2 value close to 1.0 show a very high correlation between the experimental and the predicted values of the volumetric mass transfer coefficient of CO_2. From the foregoing, it is vivid that the quadratic regression model presents a clear explanation of the relationship between the independent factors and response.

The adequacy of the quadratic regression model was ascertained between the experimental data and the model response with the diagnostic plot shown in Figure 3.

It can be observed that the quadratic regression model fits realistically, thereby adequately expressing the experimental range studied. The actual value of volumetric mass transfer coefficient represents the measured result for each experimental run, while the predicted value is evaluated from the independent variables in the regression model. The normal plot of residuals of the developed model is shown in Figure 4. It is obvious that the residuals reflect a normal distribution since virtually all the points follow a straight line curve. It is also revealed that no further improvement can be done to the model by making changes to the response because the data points are scattered and do not exhibit an S-shaped curve [24].

The graphs and tables thereby suggest that the model in Equation 2 can be regarded as the best possible model of the historical data RSM design of the $k_L a$ of CO_2 for microalgae cultivation in stirred tank bioreactors. Therefore, they shall be utilized in deriving the optimum values of the operational parameters.

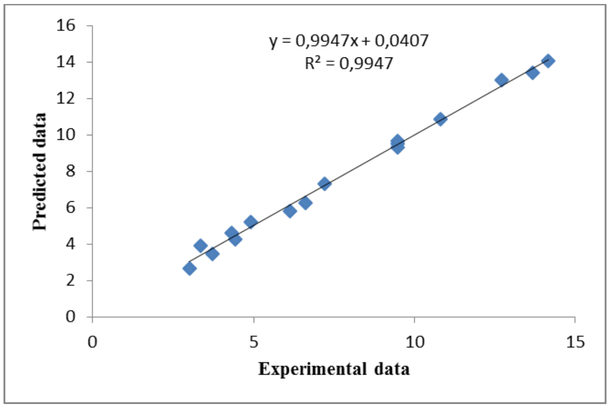

Figure 3. Cross plot between the experimental and predicted Values

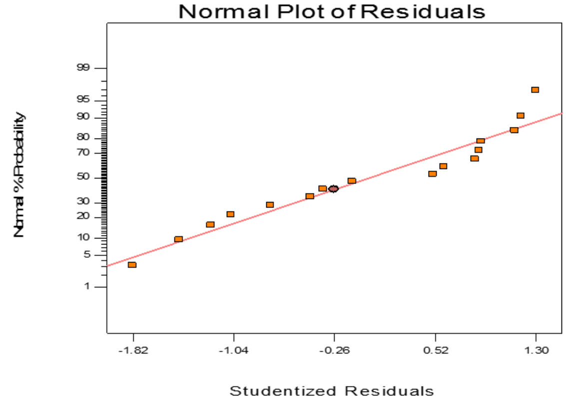

Figure 4. Normal plot of residuals for the model

3.6. Verification of optimization results

Figure 5 illustrate the predicted optimum conditions and the response studied in this work. The predicted optimum operating parameters influencing the $(K_L a)_{CO_2}$ for microalgae cultivation in stirred tank bioreactors was estimated to be agitation rate (391.33 rpm), gas flowrate (1100.32 ml/min) and gas hold up (0.05) as shown in Figure 5.

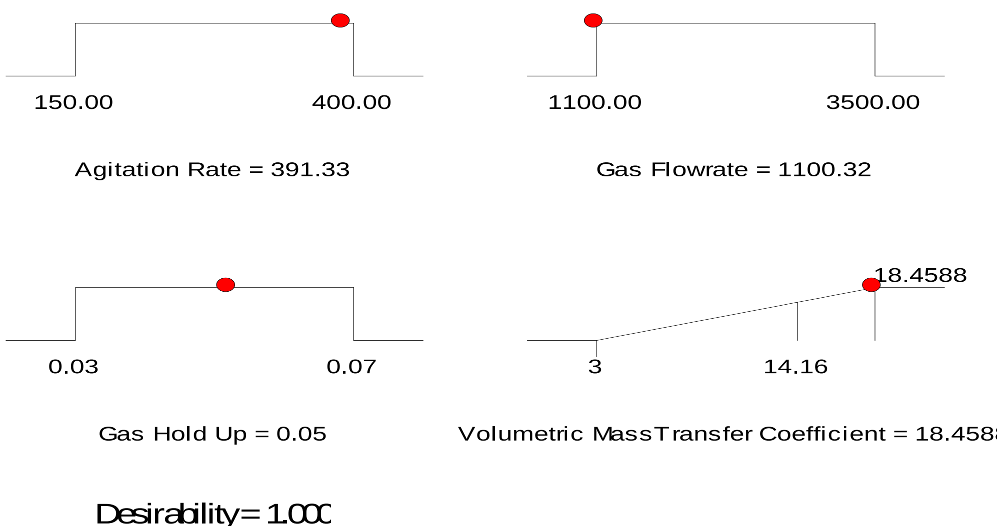

Figure 5: Optimum Conditions and Response

At these optimum conditions, the corresponding predicted optimum $k_L a$ was found to be 18.4588 h^{-1}. Experimentally, agitation rate (400 rpm), gas flowrate (1100 mL/min) and gas hold up (0.05) were optimum values of the operating parameters whose combining effect gave maximum $(K_L a)_{CO_2}$ in stirred tank bioreactors as 18.4588 h^{-1}. The experimental and predicted optimum conditions are in good agreement at desirability of 1.000. Thus, it is evident that the historical data RSM design is an efficient statistical technique for predicting the optimum operating variables for the maximization of the $(K_L a)_{CO_2}$ in stirred tank bioreactors by incorporating all factors under consideration.

4. Conclusion

This study revealed the effectiveness of RSM to successfully develop a suitable empirical model for the prediction of $(K_L a)_{CO_2}$ for microalgae cultivation. The empirical quadratic regression model has an advantage over the previously developed mechanistic models because it directly applies the factors under consideration with the aim of studying their interactive effects in contrast to the latter which requires experimental determination of secondary factors which only gives estimations of k_La and hence CO_2 consumption by microalgae. The proximity of R^2 (0.9947) and Adj-R^2 (0.9901) to 1.0 proved that there was reasonable agreement between the experimental and predicted response. The 3D response surface plots employed in explaining the effects of interaction of the operating parameters considered in this study revealed that gas hold up is the most significant factor influencing the $(K_L a)_{CO_2}$ in the bioreactors. Numerical optimization showed that the predicted optimum operating parameters observed at agitation rate (391.33 rpm), gas flowrate (1100.32 mL/min) and gas hold up (0.05) in order to achieve maximum k_La of 18.4588 h^{-1} were close to the experimental optimum conditions 400 rpm agitation rate, 1100 ml/min gas flowrate, 0.05 gas hold up and 18.4588 h^{-1} k_La. The decrease in agitation rate in the predicted optimum conditions is helpful in minimizing power consumption. It can thus be concluded that historical data RSM is a reliable statistical technique for predicting and optimizing the k_La of CO_2 for microalgae cultivation in stirred tank bioreactors.

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