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APPLICATION OF RESPONSE SURFACE METHODOLOGY (RSM) AND ARTIFICIAL NEURAL NETWORK (ANN) FOR ACHIEVING DESIRE BA IN THE BIOTRANSFORMATION OF BENZALDEHYDE USING FREE CELLS OF SACCHAROMYCES CEREVISAE AND THE EFFECT OF B-CYCLODEXTRIN

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ABSTRACT

This work dwells on the production of benzene alcohol (BA) from the biotransformation of benzaldehyde using free cells of Saccharomyces cerevisae and effects of β-Cyclodextrin. Meanwhile, the properties of BA produced was evaluated. The effects of five variables considered in this research work were evaluated using RSM and ANN. The root mean square error, the coefficient of determination, the adjusted coefficient of determination and the predicted values were used to compare the performance of the RSM and ANN models. The RMSE and R^2 of RSM and ANN were 2.00 and 0.0739; 0.9898 and 0.99206, respectively. The R^2 adj. and the predicted values of RSM and ANN were found to be 0.98416 and 0.9889 and 327.259 mg/100 ml and 351.50 mg/100 ml. The quality of BA showed that at room temperature, BA was colourless liquid with density 1.030 kg/dm^3, the boiling point and refractive index was found to be 204 ± 2 ^{0}C and 1.5453, respectively. The results indicated the ANN model to have higher predictive capability than RSM model. Thus, the ANN methodology presents a better alternative than the RSM model. The quality of produced BA was found to be in line with Analytic grade values.

Keywords: Biotransformation, Saccharomyces cerevisae, Optimization, Response surface methodology (RSM), Artificial neural network (ANN), Benzene alcohol.

1. INTRODUCTION

The use of artificial neural networks (ANNs) in the field of pharmaceutical development and optimization of the dosage forms has become a topic of discussion in the pharmaceutical literature (Kesavan and Peck, 1996; Takahara *et al.*, 1997; Chen *et al.*, 1999; Takayama *et al.*, 1999; Wu *et al.*, 2000). Compared with classical (base rule) modeling techniques, such as response surface methodology (RSM), ANNs show superiority as a modeling technique for data sets showing non-linear relationships, and thus for both data fitting and prediction abilities (Bourquin *et al.*, 1997a; Bourquin *et al.*, 1998a; 1998b).

In addition, ANNs are useful when exact mathematical information is not available. Another advantage of a model over a rule based model is that, if the process under analysis changes, new data can be added and the neural network can be trained again. In short, the whole model or rules is much stress free. ANN is a learning system based on a computational technique that can simulate the neurological processing ability of the human brain and can be applied to quantify a non-linear relationship between connecting factors and pharmaceutical responses by means of iterative training of data obtained from a designed experiment (Achanta *et al.*, 1995).

More so, in ANN, the arriving signals, called inputs, multiplied by the connection weights are first combined and then passed through a transfer function to produce the output for that neuron. The transfer function acts on the weighted sum of the neuron's inputs and the most commonly used transfer function are the Tanh and sigmoid functions (Adepoju *et al.*, 2013). The connection of neurons to one other has a significant impact on the operation of the ANN formula (feedback and feed-forward connection). Response Surface Methodology (RSM) with its allied designs such as Box-Behnken, Plackett-Burman, Central Composite Rotatable Design (CCRD) etc. helps to measure the interactions among one or more observed responses and the variable factors. If more than five factors are involved, then two-level factorial screening design will be needed. At least, some of the factors for RSM must be quantitative, continuous variables.

The aim is to find the maximum, minimum or an area where the observe respose is stable over the variable factors is tatanmour to location in the design space. Meanwhile, many of the designs can handle up to 50 numeric factors, plus up to 10 additional categorical factors. The model offers several designs depending on the number of design factor.

Both RSM and ANN strategies are suitable for biotransformation, but differ in their extrapolation and interpolation capabilities on complex non-linear biotransformation processes, and thus potentially conflict in their predictive accuracy. This paper explores and compares the capabilities of RSM and ANN in biotransformation of benzaldehyde to benzene alcohol (BA) by free cells of *saccharomyces cerevisae* in and the effects of β-cyclodextrin on cell weight, incubation time, acetaldehyde concentration, benzaldehyde concentration and β-CD level. The quality of benzene alcohol (BA) produced was also determined.

2. MATERIAL AND METHODS

2.1. Materials

All chemicals used such as; diethyl ether, anhydrous sodium sulphate, benzaldehyde, acetaldehyde, β- cyclodextrin were of analytical grade and need no further purification.

2.2. Methods

2.2.1. Microorganisms

The microorganisms employed in this study was culture locally.Meanwhile, the cutured medium was steadily maintained on a medium containing 0.004 of dextrose, 0.01 of yeast extract, 0.01 of malt extract, and 0.02 of agar at pH 7.2 (Kalil *et al.*, 2000; Adepoju *et al.*, 2013).

2.2.2. The Growing Medium

The growth medium for *Saccharomyces cerevisae* contained glucose 2%, peptone 2%, yeast extract 1% and had pH 5.5 (Long *et al.*, 1989; Adepoju *et al.*, 2013).

2.2.3. Culture Growth

Suspension of cells (1 ml) of the isolate *Saccharomyces cerevisae* containing 10^6 cells was inoculated into 9 ml of growth medium and incubated on a rotary shaker at 30 ± 2^{o}C at 240 rpm for 24 h. The obtained culture was inoculated into 100 ml of the same medium and allowed to grow for 24 h. Under the same conditions, cells were harvested by centrifuging at 10, 000 rpm for 15 min at 15 ^{o}C. The biomass obtained was washed with water, centrifuged and was used for biotransformation studies.

2.2.4. Biotransformation of Benzaldehyde to BA

The medium containing containing 5% glucose, 0.6% peptone and had pH 4.5 (Biotransformation medium-100 ml)) was inoculated with a known weight of biomass obtained. The reactor was incubated on a shaker at 30 ^{o}C and 240 rpm at different time range for adaptation of cells to the medium. Benzaldehyde and acetaldehyde was added and flasks were incubated again on a shaker at 30 ^{o}C and 240 rpm for the biotransformation process.

2.2.5. Effect of β -Cyclodextrin Addition on Biotransformation of Benzaldehyde

Effect of various levels of β-cyclodextrin was studied at benzaldehyde and acetaldehyde levels ranging from 500 mg to 1600 mg/100 ml and 400 µl to 1300 µl/100 ml, respectively. The reaction was allowed to take place for 3 h at 30 ± 2^{o}C and 240 rpm. To study the effect of β-CD level, concentration of β-CD was optimized in the range of 0.4 to 3.2%. Semi-continuous feeding of different levels of benzaldehyde and acetaldehyde was also carried out according to design expert software (Table 1).

2.3. Analysis of BA

Subsequently, the medium was centrifuged at 10,000 rpm for 15 min. The supernatant were extracted in 3:1 volumes of diethylether. The collective extract was dried over anhydrous sodium sulphate and concentrated over a temperature controlled water bath. The residue obtained was dissolved in methanol and prepared for gas chromatography (GC) analysis.

2.4. Gas Chromatography Analysis

GC model conditions used was Chemito-8510 with Oracle -1 computing integrator. A 4 meter long column of 5% OV-17 was used. The injector temperature and detector temperature (FID) was maintained at 250 ^{o}C. Column programming was as follows: 75 ^{o}C for 3 min, then 10 ^{o}C/ 1 min up to 250 ^{o}C and holding time was for 5 min. Retention times of BA was 13 min. The concentration of the compound was determined using peak area method (Adepoju *et al.*, 2013). The experiment was carried out three more time and the average means was evaluated.

2.5 Experimental Design using CCRD

2.5.1. Response Surface Analysis and Optimization

A five levels five factors Central Composite Rotatable Design (CCRD) was generated with the Design Expert 8.0.3.1 software and was employed to evaluate the interaction of various factors on BA production using free cells of *Saccharomyces cerevisae.* Five factors, namely cell weight g (wet. wt): X_1, incubation time (min): X_2, Acetaldehyde conc. (mg/100 ml): X_3, benzaldehyde conc. (mg/100 ml): X_4 and β-CD level (%): X_5 were considered (Table 1).

Table-1. Variable Factor of Central Composite Rotatble Design

Variable	Symbol	Coded factor levels				
		-2	-1	0	1	2
Cell weight (wet. wt)	X_1	2	3	4	5	6
Incubation time (min)	X_2	40	50	60	70	80
Acetaldehyde conc. (µg/100 ml)	X_3	400	700	1000	1300	1600
Benzaldehyde conc. (mg/100 ml)	X_4	500	700	900	1100	1300
ß-CD level (%)	X_5	0.4	0.8	1.2	1.6	3.2

According to the design, 50 experimental runs were generated. Each run represents a unique combination of factors levels. The total amount of BA produced was determined.

2.5.2. Artificial Neural Network (ANN)

A Neural Network Toolbox 8.0 software was used for simulation work. Experimental data generated from CCRD were used to construct the ANN module. The ideal was to use the data that are statistically well distributed in the input search window. A total number of 50 experimental data were divided into sets, 70% in training set, 15% in the validation set and 15% in the test set.

The Tanh transfer function at hidden layer and a linear transfer function at output layer were used. Research showed that the same transfer function has been used. All the factor variables and

the observe response were regulated between 0 and 1 for the reduction of network error and higher standardized results.

2.6. Statistical Data Analysis

2.6.1. Response Surface Methodology

The data obtained from biotransformation of benzaldehyde to BA was analysed statistically using response surface methodology (CCRD), so as to fit the quadratic polynomial equation generated by the Design Expert Software. In order to compare the observe response variable to the independent factor variables, multiple regressions were used to fit the coefficient of the polynomial model of the observe response. The quality of the fit of the model was evaluated using test of significance and analysis of variance (ANOVA). The fitted quadratic response model is described by Eqn 1:

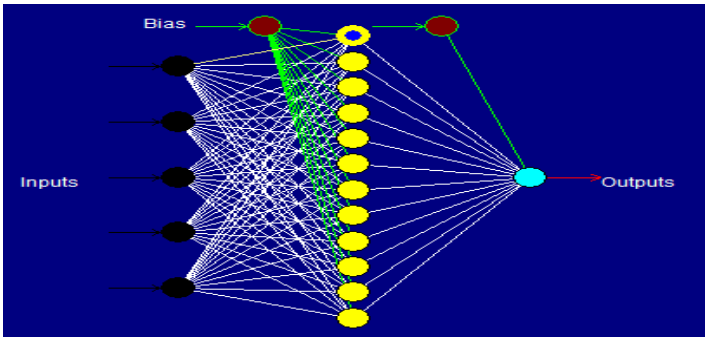
$$Y = b_0 + \sum_{i=1}^{k} b_i X_i + \sum_{i=1}^{k} b_{ii} X_i^2 + \sum_{i<j}^{k} b_{ij} X_i X_j + e \quad (1)$$

Where: Y is L-Phenylacetylcarbinol yield (response factor), b_o is the intercept value, b_i (i= 1, 2, k) is the first order model coefficient, b_{ij} is the interaction effect, and b_{ii} represents the quadratic coefficients of X_i, and e is the random error.

2.6.2. Artificial Neural Network (ANN)

Since the performance of ANN is heavily influenced network structure, hence, the learning algorithms employed was QuickProp (QP), multilayer connection type used was multilayer normal feed forward (MNFF), three total layer numbers was used and the node number of input layer was five. For the output layer, Node Number was 1, the transfer function was Tanh and the slope of transfer function and the hidden Layer was 1, the node number was 12, transfer function was also Tanh and slope of transfer function was also 1 (Fig. 1). Meanwhile, the optimum ANN structure was determined first using root mean square error (RMSE) approach. The higher coefficient R^2 was also determined; the variable analysis also was conducted to study the effects of variables towards the L-phenylacetylcarbinol production using 3D curvature's surface plots. A hybrid ANN model was used in conducting process optimization.

Fig-1. Network Structure with Twelve Transfer Functions



3. RESULTS AND DISCUSSION

3.1. Response Surface Methodology

Table 2 shows the coded factors considered in this study with BA yield, and the predicted values obtained. Design Expert 8.0.3.1 software was employed to evaluate and determine the coefficients of the full regression model equation (Eqn. 1) and their level of numerical implication. Depict in Table 3 are the results of test of significance for every regression coefficient. Considering the large F-values and low corresponding p-values, all the model terms are remarkably significant and have very strong effects on the L-PAC yield witt p< 0.05. Nevertheless, the linear term X_2^2 with F-value of 42611.15 with p-value of <0.0001, indicated the most significant model term.

Table 4 reflected the second-order response surface model results. The model F-value (terms used to estimate effects) of 29283.62 with low p-value (<0.0001) reflected a high significance for the regression model. Coefficient of determination (R^2), was employed to check the goodness of fit . It should be noted that R^2 should be at least 0.80 for the good fit of a model. Observation in this study showed that the R^2 value of 0.9898 was obtained which indicated that the sample variation of 98.98% for the BA production is attributed to the independent factors (cell weight, incubation time, acetaldehyde concentration, benzaldehyde concentration and β-CD level). The value of the adjusted determination coefficient (Adj. R^2) was 0.98416 and all p-values were less than 0.05, implying that the model proved suitable for the adequate representation of the actual relationship among the selected factors. The lack-of-fit term of 0.8317 was not significant relative to the pure error. The developed regression model equation describing the factors of cell weight (X_1), incubation time (X_2), acetaldehyde conc. (X_3),

Table-2. Central composite rotatable design matrix of five-level-five-factors response surface study, RSM model predicted and ANN model predicted BA

Std. run	X_1	X_2	X_3	X_4	X_5	(mg/100 ml)		
						Actual BA	RSM Predicted	ANN Predicted
1	-1	-1	-1	-1	-1	259.00	258.28	259
2	1	-1	-1	-1	-1	276.00	276.23	276
3	-1	1	-1	-1	-1	300.00	299.78	300
4	1	1	-1	-1	-1	298.00	298.10	298
5	-1	-1	1	-1	-1	309.00	309.00	309
6	1	-1	1	-1	-1	311.00	311.32	311
7	-1	1	1	-1	-1	338.00	338.12	338
8	1	1	1	-1	-1	321.00	320.82	321
9	-1	-1	-1	1	-1	273.00	273.15	273
10	1	-1	-1	1	-1	294.00	294.23	294
11	-1	1	-1	1	-1	304.00	303.53	304
12	1	1	-1	1	-1	305.00	304.98	305
13	-1	-1	1	1	-1	313.00	313.25	313
14	1	-1	1	1	-1	319.00	318.70	319
15	-1	1	1	1	-1	331.00	331.24	331
16	1	1	1	1	-1	317.00	317.07	317
17	-1	-1	-1	-1	1	305.00	305.43	305

Continue

18	1	-1	-1	-1	1	321.00	320.51	321
19	-1	1	-1	-1	1	332.00	331.81	332
20	1	1	-1	-1	1	327.00	327.26	327
21	-1	-1	1	-1	1	300.00	300.03	300
22	1	-1	1	-1	1	299.00	299.48	299
23	-1	1	1	-1	1	314.00	314.03	314
24	1	1	1	-1	1	294.00	293.85	294
25	-1	-1	-1	1	1	282.00	281.93	282
26	1	-1	-1	1	1	300.00	300.13	300
27	-1	1	-1	1	1	297.00	297.18	297
28	1	1	-1	1	1	296.00	295.76	296
29	-1	-1	1	1	1	266.00	265.90	266
30	1	-1	1	1	1	268.00	268.48	268
31	-1	1	1	1	1	269.00	268.77	269
32	1	1	1	1	1	251.00	251.73	251
33	-2	0	0	0	0	300.00	300.37	300
34	2	0	0	0	0	302.00	301.44	302
35	0	-2	0	0	0	337.00	336.69	351.5
36	0	2	0	0	0	366.00	366.12	351.5
37	0	0	-2	0	0	297.00	297.43	301.5
38	0	0	2	0	0	306.00	305.38	301.5
39	0	0	0	-2	0	320.00	320.11	320
40	0	0	0	2	0	288.00	287.70	288
41	0	0	0	0	-2	266.00	266.22	266
42	0	0	0	0	2	245.00	244.59	245
43	0	0	0	0	0	277.00	277.48	277.5
44	0	0	0	0	0	278.00	277.48	277.5
45	0	0	0	0	0	277.00	277.48	277.5
46	0	0	0	0	0	278.00	277.48	277.5
47	0	0	0	0	0	277.00	277.48	277.5
48	0	0	0	0	0	278.00	277.48	277.5
49	0	0	0	0	0	277.00	277.48	277.5
50	0	0	0	0	0	278.00	277.48	277.5

Table-3. Test of significance for all regression coefficient terms

Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**
X_1	2.20	1	2.20	9.87	<0.0001
X_2	1657.91	1	1657.91	7444.86	0.0039
X_3	121.04	1	121.04	543.52	<0.0001
X_4	2010.67	1	2010.67	9028.94	<0.0001
X_5	895.51	1	895.51	4021.32	<0.0001
X_1X_2	770.28	1	770.28	3458.96	<0.0001
X_1X_3	488.28	1	488.28	2192.64	<0.0001
X_1X_4	19.53	1	19.53	87.71	<0.0001
X_1X_5	16.53	1	16.53	74.23	<0.0001
X_2X_3	306.28	1	306.28	1375.36	<0.0001
X_2X_4	247.53	1	247.53	1111.54	<0.0001
X_2X_5	457.53	1	457.53	2054.55	<0.0001
X_3X_4	225.78	1	225.78	1013.88	<0.0001
X_3X_5	6300.03	1	6300.03	28290.42	<0.0001

Continue

X_4X_5	2945.23	1	2945.23	13225.84	<0.0001
X_1^2	952.62	1	952.62	4277.77	<0.0001
X_2^2	9489.13	1	9489.13	42611.15	<0.0001
X_3^2	993.73	1	993.73	4462.36	<0.0001
X_4^2	1212.29	1	1212.29	5443.80	<0.0001
X_5^2	846.47	1	846.47	3801.08	<0.0001

Table-4. Analysis of variance (ANOVA) of regression equation

Source	Sum of Squares	df	Mean Square	F-value	p-value
Model	29283.62	20	1464.18	6574.93	<0.0001
Residual	6.46	29	0.22		
Lack of fit	4.46	22	0.20	0.71	0.7490
Pure error	2.00	7	0.29		
Cor total	29290.08	49			
			R^2 = 98.98%,	R^2 (adj.) = 98.42%	

benzaldehyde (X_4) and β-CD level (X_5) and their respective interactions is described in Eqn. (2).

$$Y_2(mg/100\ ml) = 277.48 + 0.23x_1 + 6.19x_2 + 1.67x_3 - 6.81x_4 - 4.55x_5 - 4.91x_1x_2 - 3.91x_1x_3 + 0.78x_1x_4 - 0.72x_1x_5 - 3.09x_2x_3 - 2.78x_2x_4 - 3.78x_2x_5 - 2.66x_3x_4 - 14.03x_3x_5 - 9.59x_4x_5 + 4.14x_1^2 + 13.07x_2^2 + 4.23x_3^2 + 4.67x_4^2 - 3.90x_5^2 \quad (2)$$

Where $Y_.$ = BA yield (mg/100 ml)

The model coefficients and probability coded values are shown in Table 5. The low values of standard error observed in the intercept and all the model terms showed that the regression model fits the data well, and the prediction is good. The variance inflation factor (VIF) obtained in this study showed that the 8-centre points are orthogonal to all other factors in the model. The model also exhibited suitable for the adequate depiction of the real connection among the selected independent factors.

Usually, the three-dimensional (3D) response surface plots are graphical representations of the regression equation for the optimization of the reaction variables, and they are represented in Fig. 2. The curvatures' nature of 3D surfaces in Figure 2a, b, e, f, g, and i suggested reciprocal interaction of cell weight with incubation time, cell weight with acetaldehyde conc., incubation time with acetaldehyde conc., incubation time with benzaldehyde conc. incubation time with β-CD level and benzaldehyde conc. with β-CD level, respectively. Meanwhile, the nature of curvatures' of 3D surfaces in Figure 2c, d, h, j showed moderate interactions of cell weight with benzaldehyde conc., cell weight with β-CD level, acetaldehyde conc. with benzaldehyde conc., and acetaldehyde conc. with β-CD level, respectively. The optimum values of the independent factors selected for the biotransformation of benzaldehyde to BA were obtained by solving the regression equation (Eq. 2) using the Design-Expert software package. The optimum conditions for this process were statistically predicted as X_1 = 6.0 g (wet. wt.), X_2 = 80 (min), X_3 = 400.00 (µg/100 ml), X_4 = 500

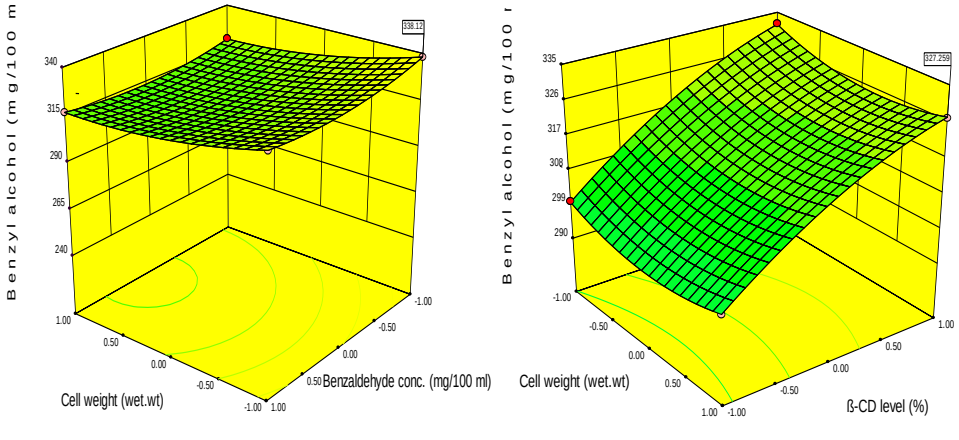
(mg/100 ml) and X_5 = 3.20 %. The predicted BA yield under the above set conditions was 327.259 (mg/100 ml). In order to verify the prediction of the model, the optimum conditions were applied to three independent replicates, and the average BA yield obtained was 326.00 (mg/100 ml), which was well within the range predicted by the model equation.

Table-5. Regression coefficients and significance of response surface quadratic

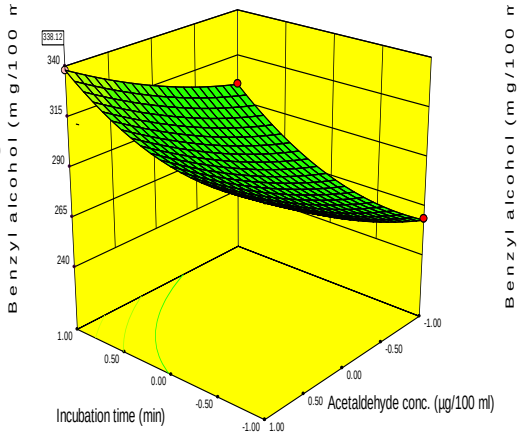
Fact.	Coefficient estimate	df	Standard error	95% CI Low	95% CI high	VIF
Intercept	277.48	1	0.17	277.15	277.82	-
X_1	0.23	1	0.072	0.079	0.37	1.00
X_2	6.19	1	0.072	6.04	6.33	1.00
X_3	1.67	1	0.072	1.53	1.82	1.00
X_4	-6.81	1	0.072	-6.96	-6.67	1.00
X_5	-.4.55	1	0.072	-4.69	-4.40	1.00
X_1X_2	-4.91	1	0.083	-5.08	-4.74	1.00
X_1X_3	-3.91	1	0.083	-4.08	-3.74	1.00
X_1X_4	0.78	1	0.083	0.61	0.95	1.00
X_1X_5	-0.72	1	0.083	-0.89	-0.55	1.00
X_2X_3	-3.09	1	0.083	-3.26	-2.92	1.00
X_2X_4	-2.78	1	0.083	-2.95	-2.61	1.00
X_2X_5	-3.78	1	0.083	-3.95	-3.61	1.00
X_3X_4	-2.66	1	0.083	-2.83	-2.49	1.00
X_3X_5	-14.03	1	0.083	-14.20	-13.86	1.00
X_4X_5	-9.59	1	0.083	-9.76	-9.42	1.00
X_1^2	4.14	1	0.063	4.01	4.27	1.05
X_2^2	13.07	1	0.063	12.94	13.20	1.05
X_3^2	4.23	1	0.063	4.10	4.36	1.05
X_4^2	4.67	1	0.063	4.54	4.80	1.05
X_5^2	-3.90	1	0.063	-4.03	-3.77	1.05

Fig-2. The curvatures' nature of 3D surfaces plots for BA

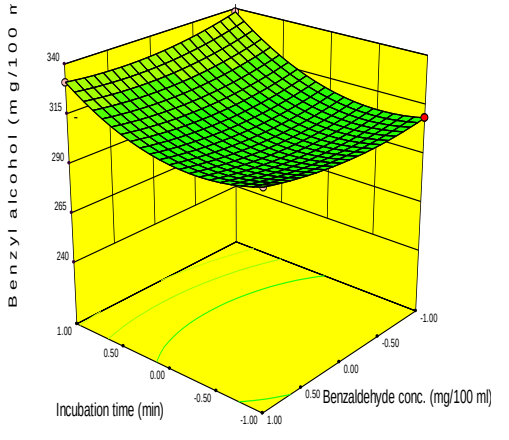
(a) (b)



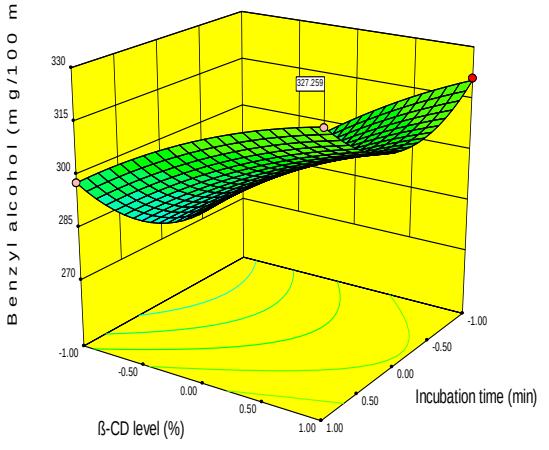
(c) (d)


Benzyl alcohol (mg/100 m
338.12
340
315
290
265
240
1.00
0.50
0.00
-0.50
-1.00
Incubation time (min)
Acetaldehyde conc. (µg/100 ml)

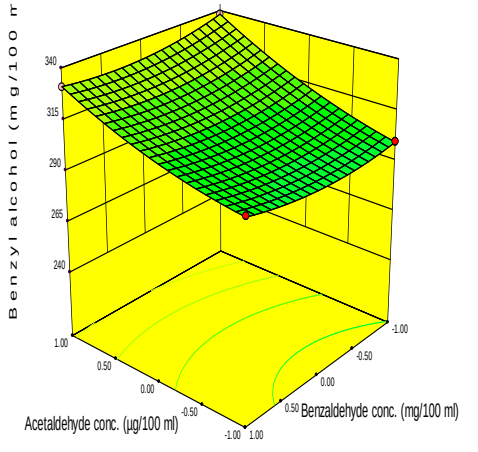
(e)


Benzyl alcohol (mg/100 m
340
315
290
265
240
Incubation time (min)
Benzaldehyde conc. (mg/100 ml)

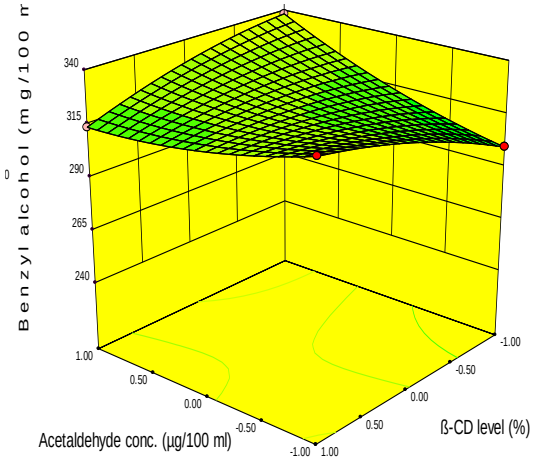
(f)


Benzyl alcohol (mg/100 m
327.259
330
315
300
285
270
ß-CD level (%)
Incubation time (min)

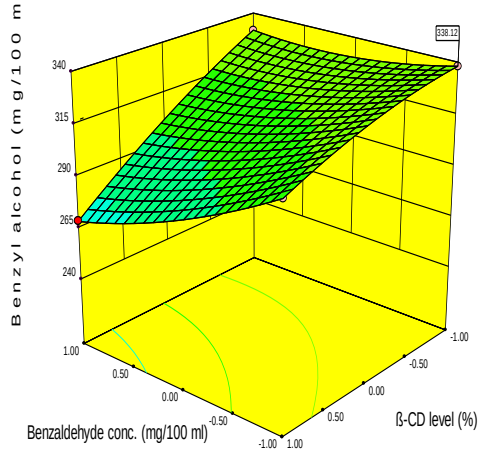
(g)


Benzyl alcohol (mg/100 m
340
315
290
265
240
Acetaldehyde conc. (µg/100 ml)
Benzaldehyde conc. (mg/100 ml)

(h)


Benzyl alcohol (mg/100 m
340
315
290
265
240
Acetaldehyde conc. (µg/100 ml)
ß-CD level (%)

(i)


Benzyl alcohol (mg/100 m
338.12
340
315
290
265
240
Benzaldehyde conc. (mg/100 ml)
ß-CD level (%)

(j)

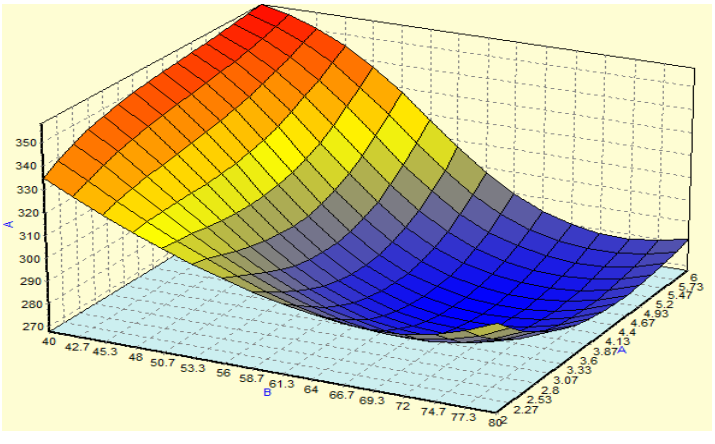
3.2. Artificial Neural Network

Depicts also in Table 2 was the observed yields as well as the difference obtained by ANN software. The effects of unexplained variability in the BA yield response due to extraneous factors were minimized by randomizing the order of experiments. The goodness of fit of the model was checked by the coefficient of determination (R^2). Again, R^2 should be at least 0.80 for the good fit of a model, Guan and Yao (2008). In this case, the R^2 value of 0.99206 indicated that the sample variation of 99.21% for the BA production is attributed to the independent factors (cell weight, incubation time, acetaldehyde concentration, benzaldehyde concentration and β-CD level). The values of RMSE and the adjusted determination coefficient (Adj. R^2) were also evaluated to be 0.0739 and 0.98416, respectively.

Generally, the three-dimensional (3D) curvature plots are graphical representations of the regression equation for the optimization of the reaction variables, and they are represented in Fig. 3. The curvatures' nature of 3D surfaces in Fig. 3a, c, d, e, f, g, j, suggested mutual reciprocal interaction of cell weight with incubation time, cell weight with benzaldehyde conc., cell weight with β-CD level, incubation time with acetaldehyde conc., incubation time with benzaldehyde conc., incubation time with β-CD level, and benzaldehyde conc. with β-CD level, respectively. On the other hand, the nature of curvatures' of 3D surfaces in Fig. 3b, h, i, indicated moderate interactions of cell weight with acetaldehyde conc., acetaldehyde conc. with benzaldehyde conc., and acetaldehyde conc. with β-CD level, and, respectively.

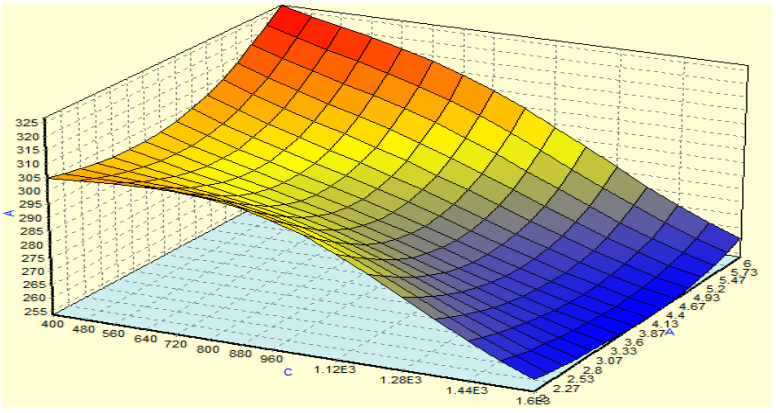
The predicted BA yield under the above set conditions was 351.50 (mg/100 ml). In order to verify the prediction of the model, the optimal conditions were also applied to three independent replicates, and the average BA yield obtained was 351.00 (mg/100 ml), which is well within the predicted value for the model equation.

Fig-3. (a-j): 3-D curvatures' plots



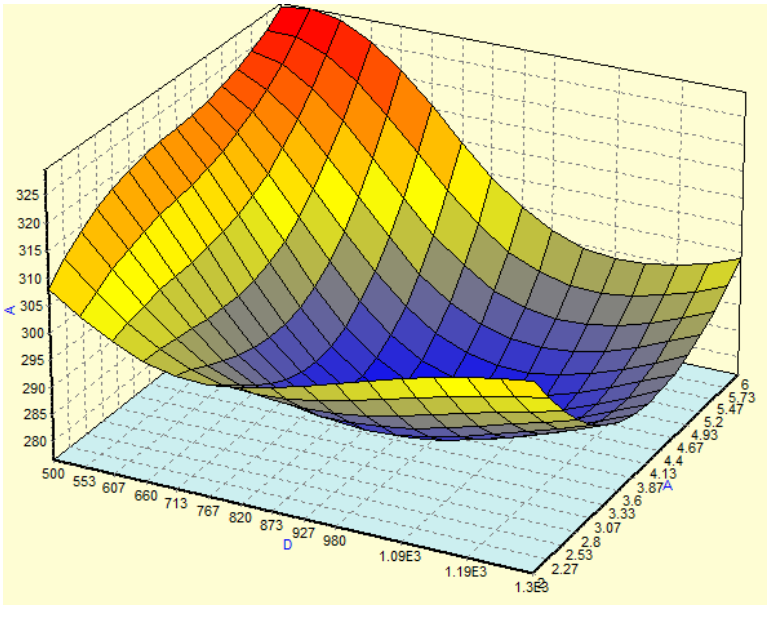
(a)

A(vertical) = BA yield (mg/100 ml), A(horizontal) = Cell weight g(wet.wt), B(horizontal) = Incubation time (min)



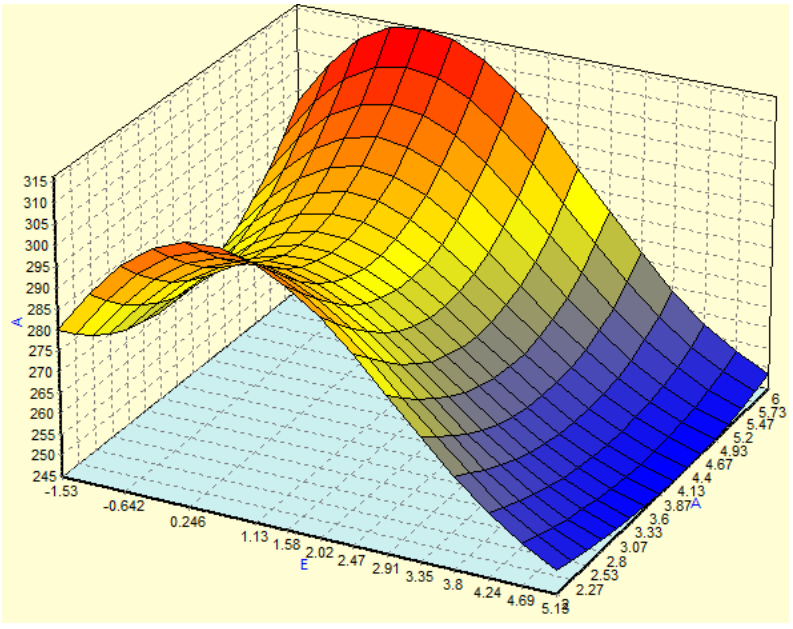
(b)

A(vertical) = BA yield (mg/100 ml), A(horizontal) = Cell weight g(wet.wt), C(horizontal) = Acetaldehyde conc. (µg/100 ml)



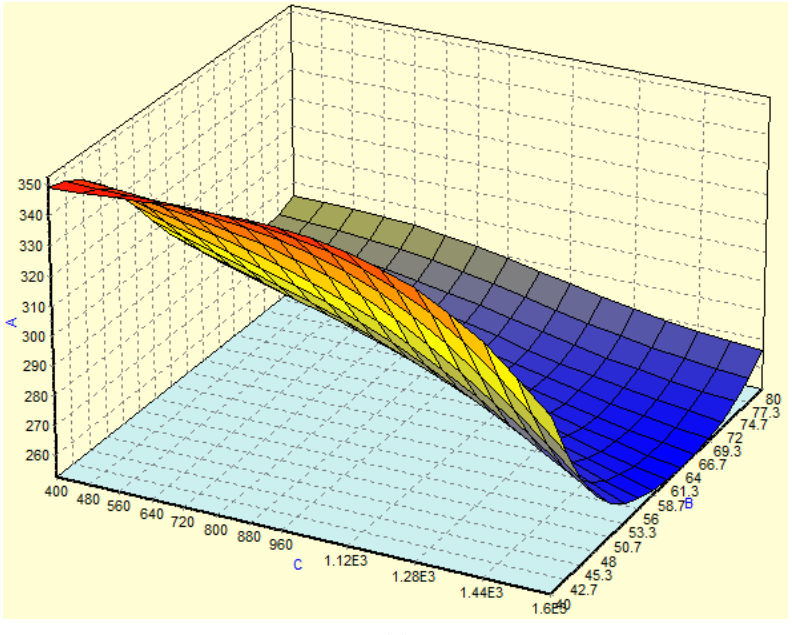
(c)

A(vertical) = BA yield (mg/100 ml), A(horizontal) = Cell weight g(wet.wt), D(horizontal) = Benzaldehyde conc. (mg/100 ml)



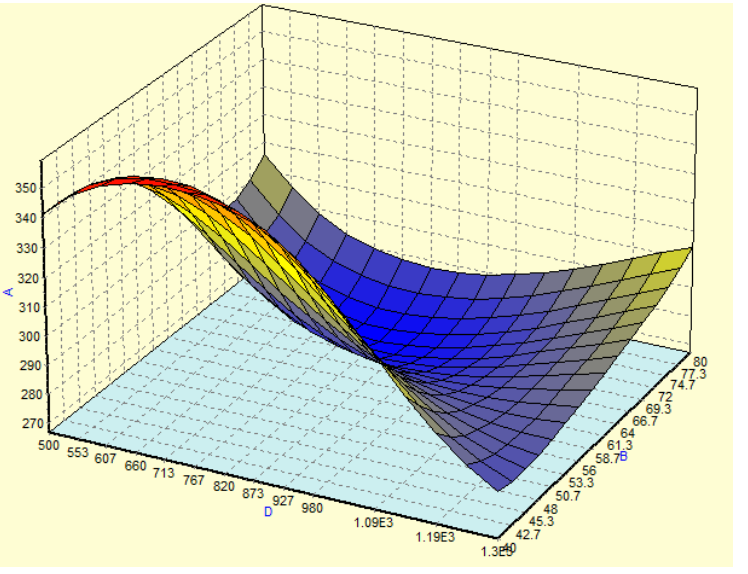
(d)

A(vertical) = BA yield (mg/100 ml), A(horizontal) = Cell weight g(wet.wt), C(horizontal) = ß-CD level (%)



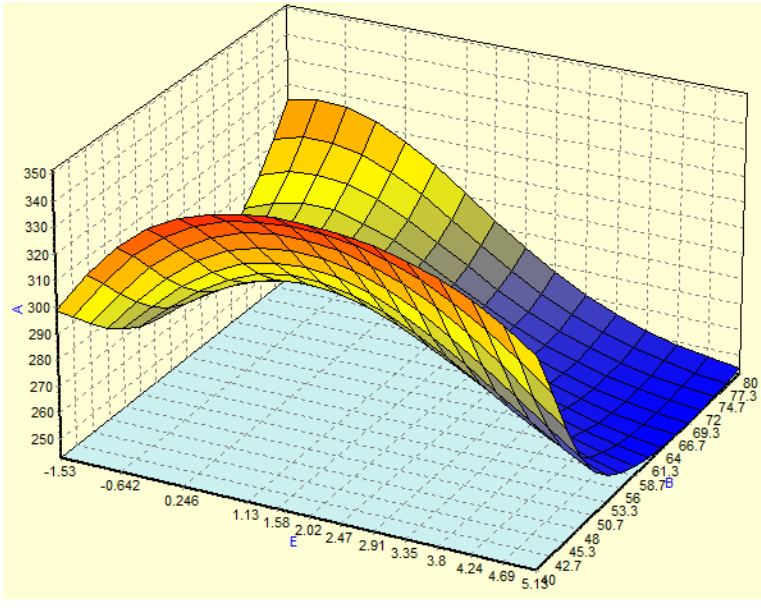
(e)

A(vertical) = BA yield (mg/100 ml), B(horizontal) = Incubation time (min), C(horizontal) = Acetaldehyde conc. (µg/100 ml)



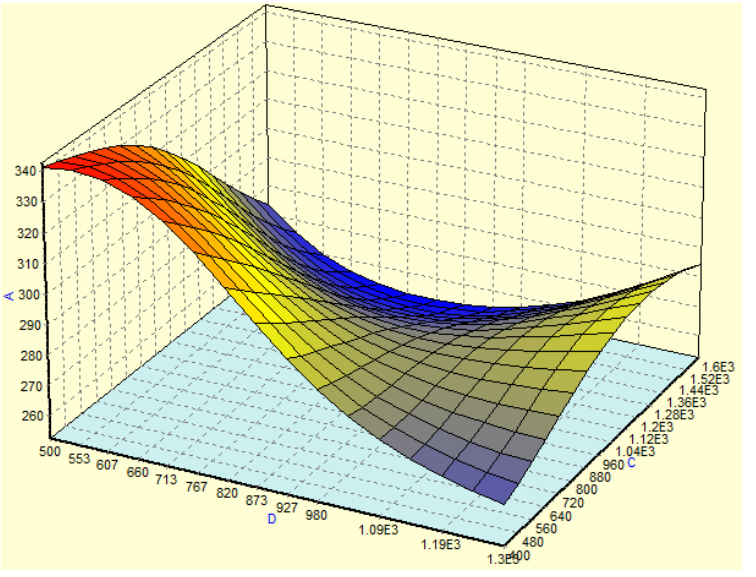
(f)

A(vertical) = BA yield (mg/100 ml), B(horizontal) = Incubation time (min), D(horizontal) = Benzaldehyde conc. (mg/100 ml)



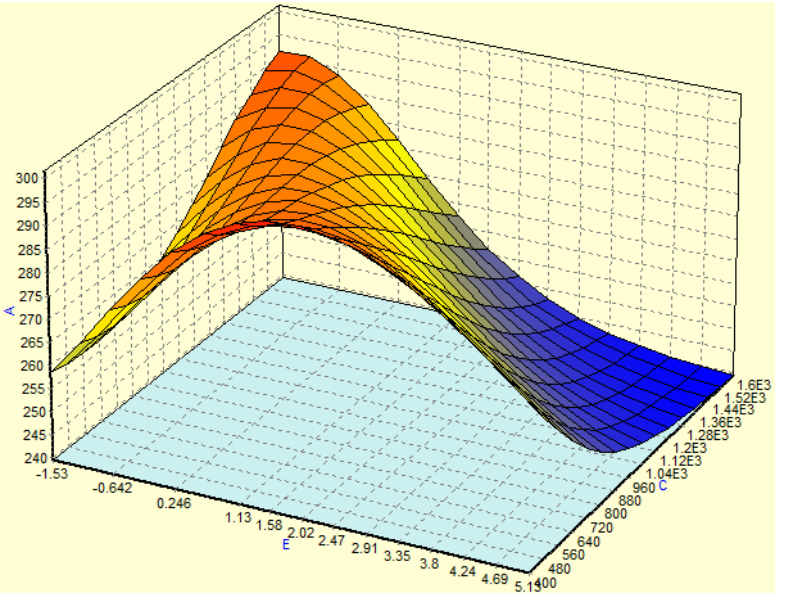
(g)

A(vertical) = BA yield (mg/100 ml), B(horizontal) = Incubation time (min), E(horizontal) = ß-CD level (%)



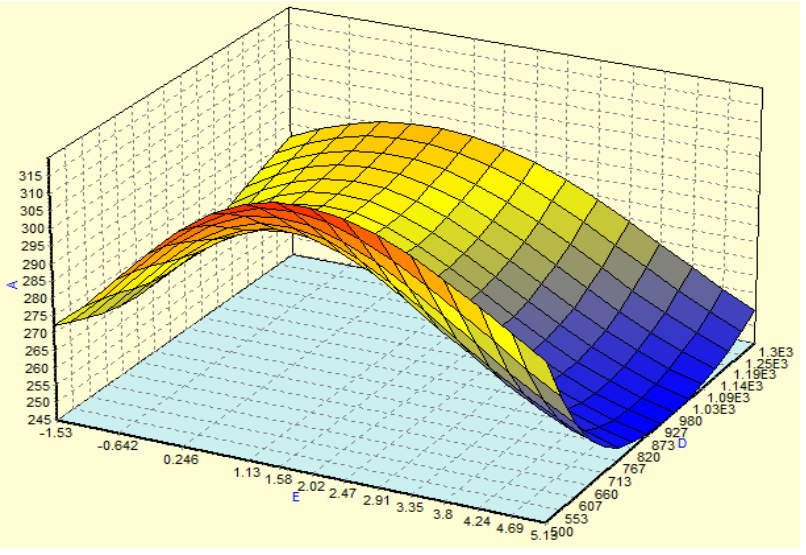
(h)

A(vertical) = BA yield (mg/100 ml), C(horizontal) = Acetaldehyde conc. (µg/100 ml), D(horizontal) = Benzaldehyde conc. (mg/100 ml)



(i)

A(vertical) = BA yield (mg/100 ml), C(horizontal) = Acetaldehyde conc. (µg/100 ml), E(horizontal) = ß-CD level (%)



(j)

A(vertical) = BA yield (mg/100 ml), D(horizontal) = Benzaldehyde conc. (mg/100 ml), E(horizontal) = ß-CD level (%).

3.3. Comparison of RSM and ANN Models

The comparison of RSM and ANN methodologies for predicted experimental results was done in terms of coefficient of determination (R^2), root mean squared error (RMSE), adjusted coefficient of determination (R^2 adj.) and the predicted yield of BA. The comparative values RSME, R^2, R^2 adj and predicted are given in Table 6. The RMSE for the design matrix by RSM and ANN are 2.00 and 0.0739, the obtained R^2 are 0.9898 and 0.99206, and the R^2 adj. are 0.98416 and 0.9889. The predicted (ANN) optimum emerged with the highest observed experimental BA production, with values above expectation (351.50 mg/100 ml). These observations raise the suggestion that ANN derived models more accurate in approximating the dynamics of BA biotransformation processes. The relatively low (327.259 mg/100 ml) predicted accuracy exhibited by RSM model in this work, suggest the inability of this modeling strategy (Although mostly used) to approximate the non linear dynamics nature of biotransformation processes, being limited by its second- order quadratic polynomial function. Meanwhile, the excellent predictive accuracy of ANN is accounted by the fact that the model class uses transfer functions in the hidden and output layers to approximate complex non-linearities in systems, thus capturing the non linear behaviour in the biotransformation process dynamics.

3.4. Qualities of BA

In order to ascertain the quality of the BA produced the content and the compositions was subjected to physical analysis test. The results obtained are shown in Table 6. At room temperature, BA was colourless liquid, the density was determined to be 1.030 kg/dm^3, meanwhile, the boiling point and refractive index was found to be 204 ± 2 ^{0}C and 1.5453, respectively.

4. CONCLUSSION

In this study, the effects of cell weight g (wet. wt): X_1, incubation time (min): X_2, Acetaldehyde conc. (mg/100 ml): X_3, benzaldehyde conc. (mg/100 ml): X_4 and β-CD level (%): X_5 were considered using RSM and ANN methods. The RMSE, R^2, R^2 adj. and the predicted values were used to compare the performance of the RSM and ANN models. The ANN model was found to have higher predictive capability than RSM model with 50 numbers of experimental runs. Thus, the ANN methodology presents a better alternative. The quality of produced BA was found to conform in line with Analytic grade.

Table-6. Comparison of RSM and ANN

Data	Values	
	RSM	**ANN**
R^2 adj.	0.98416	0.98890
RMSE	2.00	0.0739
R^2	0.9898	0.99206
Predicted (mg/100 ml)	327.259	351.50
L-PAC validated yield (mg/100 ml)	326.00	351.00

5. ACKNOWLEDGEMENT

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