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MODELING AND OPTIMIZATION OF PHENYLACETYYLCARBINOL SYNTHESIS VIA BENZALDEHYDE: A CASE OF ARTIFICIAL NEURAL NETWORK VS. RESPONSE SURFACE METHODOLOGY

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ABSTRACT

In this study, a comparative optimization of biotransformation of benzaldehyde to L-Phenylacetylcarbinol via free cells of Saccharomyces cerevisae using Response Surface Methodology (RSM) and Artificial Neural Network (ANN) was done. A polynomial regression model was developed and RSM optimum process was determined. In developing ANN model, performance of ANN is heavily influenced by its network structure, five-level-five-factors design was applied, which generated 50 experimental runs from CCD design of RSM. The inputs for the ANN were cell mass (wet. wt), incubation duration (min), concentration of acetaldehyde (mg/100 ml), concentration of benzaldehyde (mg/100 ml), and β-cyclodextrin level (%): X_5. The learning algorithms used was QP with MNFF and the transfer function was Tanh. The RMSE, R^2, AAD and predicted values were used to compare the performance of the RSM and ANN models. The extrapolative fitness of ANN model was found to be higher than RSM extrapolative fitness model. Thus, it can be concluded that even though RSM is mostly used method for experimental optimization, the ANN methodology present a better alternative.

Keywords: Biotransformation, *Saccharomyces cerevisae*, Response surface methodology (RSM), Artificial neural network (ANN), L- phenylacetylcarbinol.

Contribution/ Originality

This study contributes in the existing literature to science and engineering. This study uses new estimation methodology for the conversion of benzaldehyde to L-PAC. This study originates new formula to enhance the concentration of L-PAC. This study is one of very few studies which have investigated the use of β-CD to improve the L-PAC formation. The paper contributes the first logical analysis in modeling and optimization of L-PAC formation. The paper's primary

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contribution is finding that L-PAC production can be enhanced using statistical software. This study documents the superiority of artificial neural network over response surface methodology.

1. INTRODUCTION

L- Phenylacetylcarbinol (1-hydroxy-1-phenyl-2-propanone or 1-hydroxy-1-phenylacetone or α-hyroxybenzyl methyl ketone) is one of the mostly used biotransformation product, produced almost absolutely by *Saccharomyces cerevisae*, *Candida utilis* or *Torulaspora delbrueckii*. It is used as the key intermediate for the synthesis of most pharmaceutical products, such as pseudoephedrine, L- ephedrine, nor-pseudoephedrine and norephedrine [1, 2]. The demand for industrial application of L- Phenylacetylcarbinol through the biotransformation of benzaldehyde

Table: Nomenclatures

Nomenclatures	
X_1	Cell mass (wet. wt)
X_2	Incubation duration (min)
X_3	concentration of acetaldehyde (µg/100 ml)
X_4	concentration of benzaldehyde (mg/100 ml)
X_5	ß-cyclodextrin level (%)
X_1X_2	Cross product between cell mass and Incubation duration
X_1X_3	Cross product between cell mass and concentration of acetaldehyde
X_1X_4	Cross product between cell mass and concentration of benzaldehyde
X_1X_5	Cross product between cell mass and ß-cyclodextrin level
X_2X_3	Cross product between Incubation duration and acetaldehyde concentration
X_2X_4	Cross product between Incubation duration and benzaldehyde concentration
X_2X_5	Cross product between Incubation duration and ß-cyclodextrin level
X_3X_4	Cross product between concentration of acetaldehyde and concentration of benzaldehyde
X_3X_5	Cross product between concentration of acetaldehyde and ß-cyclodextrin level
X_4X_5	Cross product between concentration of benzaldehyde and ß-cyclodextrin level
Greek Symbols	
µg	Microgram
β-cyclodextrin	Beta-cyclodextrin
β_o	Intercept value
β_1	linear coefficients
β_{12}	Interaction coefficients
β_{11}	Quadratic coefficients
ε	Error term
Abbreviations	
Mg	Milligram
ANN	Artificial neural network
RSM	Response surface methodology
DOE	Degree of experiment

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Rpm	Revolution per minute
GC	Gas chromatograph
CCD	Central composite design
Y	L-PAC concentration
L-PAC	L-phenylacetylcarbinol
QP	QuickProp
MNFF	Multilayer normal feed forward
RMSE	Root mean square error
3D	3-Dimensional
R^2	Sample correlation coefficient
Adj. R^2	Adjusted sample correlation coefficient
df	Degree of freedom
wet. wt	Wet per weight
VIF	Variance inflation factor
CI	Confidence level
AAD	Average absolute deviation

came into been when the experiment was first carried out by [2]. The demand for L-phenylacetylcarbinol is increasing faster than its production and hence economical process models are needed. However, the formation of bi-products such as Pac-diol, phenyl methanol and residual benzene, along the production of L-Pac from benzaldehyde under normal fermentative conditions using yeast shows that the qualitative conversion conversion has never been achieved [1]. The use of modeling and optimization by earlier researcher has shown that statistical analysis can improved the yield of the product by minimizing the control variables.

Presently, modeling and optimization are important aspects in the biotransformation process [1, 2]. Single variable optimization method (conventional optimization) is not only time-consuming and tiresome but also unable to describe the complete effects of the parameters in the process and ignores the interactions of results [3, 4]. Classical modeling techniques, such as response surface methodology (RSM) with its allied designs such as Box-Behnken, Plackett-Burman, Central Composite Rotatable Design, Factorial design and artificial neural networks (ANN) are fast and consistent methods by decreasing the total number of experiments, fixing short lists significant factors and process by regarding the reciprocal interactions among the variables factor and to give an estimate of the combined effects on these variables.

RSM is a collection of statistical techniques for designing experiments, building models, evaluating the effects of factors and searching for the optimum conditions [5]. The experimental responses to design of experiments (DOEs) are fitted to a quadratic function in RSM [6]. The models assumed a second-order polynomial relation can reasonably approximate many of the biotransformation process dynamics. ANNs are artificial learning tool for optimization [7]. Its power resides on its capability to learn from historical process data and approximate linear and non-linear functions [8].

RSM as well as ANN approaches are appropriate for biotransformation; though vary in their extrapolation and interpolation competences on multifaceted non-linear biotransformation progressions which brought about conflict in their analytical accurateness. This paper explores

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and compares the capabilities of RSM and ANN in biotransformation of benzaldehyde to L-Phenylacetylcarbinol by free cells of *saccharomyces cerevisae* in the presence of β-cyclodextrin on cell weight, incubation time, acetaldehyde concentration, benzaldehyde concentration and β-cyclodextrin level. Moreover, the optimized conditions are further validated experimentally.

2. EXPERIMENTAL PROCEDURES

2.1. Materials and Methods Used

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

2.1.1. Microbes

S. cerevisiae used in this work was isolated locally. The culture was steadily kept on a medium comprising 0.004 dextrose, 0.01 yeast extract, 0.01 malt extract, and 0.02 agar at pH 7.2 [1, 5].

2.1.2. The Growing Medium

The growth medium for *S. cerevisae* contained glucose 0.02, peptone 0.02, yeast extract 0.01 and had pH 5.5 [1, 9].

2.1.3. Culture Growing

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

2.1.4. Biotransformation of Benzaldehyde to L-PAC

Biotransformation medium (100 ml) containing 0.05 glucose, 0.006 peptone and with a pH 4.5 was immunized with a known weight of obtained biomass. The apparatus was incubated on a rotatory shaker at 30 °C and 240 rpm at different time range for cells version to the medium. Benzaldehyde concentration and acetaldehyde concentration was added and the apparatus were incubated over on a shaker for the biotransformation process.

2.1.5. Effect of B -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

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reaction was allowed for 3 h. To study the effect of β-cyclodextrin level, benzaldehyde and acetaldehyde concentration was added in different ranges as design by CCD (Table 1).

Table-1. Factors and their Levels for Composite Central Design

Variable	Symbol	Coded factor levels				
		-2	-1	0	1	2
Cell mass (wet. wt)	X_1	2	3	4	5	6
Incubation duration (min)	X_2	40	50	60	70	80
Concentration of acetaldehyde µg/100 ml) x 10^2	3	4	7	10	13	16
Concentration of benzaldehyde (mg/100 ml) x 10^2	X_4	5	7	9	11	13
ß-cyclodextrin level (%)	X_5	0.4	0.8	1.2	1.6	3.2

2.3. Breakdown of Biotransformation Product

Once the biotransformation is completed, centrifugation of the medium was carried out at 10,000 rpm for 15 min. Diethyl ether was used to wash the extracted supernatant, and then dried over Na_2SO_4 and concentrated over a temperature controlled water bath. The residue obtained was dissolved in methanol and prepared for gas chromatography (GC) analysis.

2.4. GC Analysis

The dissolved residual in methanol was subjected to analysis using gas chromatography analyzer with model Chemito-8510, Oracle -1 computing integrator, 4 meter long column of 5% OV-17, the injector temperature and detector temperature (FID) was maintained at 250 ^oC. Column programming was followed using 75 ^oC for 3 min, then 10 ^oC/ 1 min up to 250 ^oC and holding time of 5 min. L-PAC retention time used was 17 min. Meanwhile, the concentration of the L-PAC was determined using peak area method [1, 10]. The experiment was validated by carried out three independent replicate until it was found to be reproducible within ± 3% limits.

2.5. Experimental Design

2.5.1. Response Surface Analysis and Optimization

Central Composite Design (CCD) of five- levels- five- variable factors were generated with the Design Expert 8.0.3.1 software and was employed to evaluate the interaction of various factors on L-phenylacetylcarbinol production using free cells of *Saccharomyces cerevisae*. Five factors, namely cell mass (wet. wt): X_1, incubation duration (min): X_2, concentration acetaldehyde (mg/100 ml): X_3, concentration benzaldehyde (mg/100 ml): X_4 and β-cyclodextrin level (%): X_5 were considered (Table 1). The CCD experimental data was divided into two: via training and testing data sets (Table 2). Thirty three (33) out of the fifty experimental data were used as training set while the remaining seventeen (17) were used as testing set.

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2.5.2. Artificial Neural Network (ANN)

Neural Power version 2.5 (CPC-X software) was used in this study. Experimental data generated via CCD were used to for 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, 35 in training set, 8 in the validation set and 7 in the test set. The Tanh transfer function at hidden layer and a linear transfer function at output layer were used. The similar transfer function has been used [11-14]. The training function selected for the network is 'Tanh'. All variables and response were normalized for the reduction of network error and higher standardized results.

2.6. Statistical Data Analysis

2.6.1. Statistical Data Analysis by Response Surface Methodology

The data obtained from biotransformation of benzaldehyde to L-phenylacetylcarbinol was analysed statistically using response surface methodology (CCD), so as to fit the quadratic polynomial equation generated by the Design Expert Software. To associate the response variable to the independent variables, multiple regressions was used to fit the coefficient of the polynomial model of the 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 Eq. (1):

$$Y = \beta_o + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 + \beta_5 X_5 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{14} X_1 X_4 + \beta_{15} X_1 X_5 + \beta_{23} X_2 X_3 + \beta_{24} X_2 X_4 + \beta_{25} X_2 X_5 + \beta_{34} X_3 X_4 + \beta_{35} X_3 X_5 + \beta_{45} X_4 X_5 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{44} X_4^2 + \beta_{55} X_5^2 + \in \quad (1)$$

Y is the value of response (actual value) corresponding to the value of X_1, X_2, X_3, X_4, X_5 of the explanatory variable, β_o is the intercept, $\beta_1, \beta_2, \beta_3$ β_4 and β_5 are the linear coefficients, β_{12}, $\beta_{13}, \beta_{23}, \beta_{14}$ and β_{15} are interaction coefficients, while $\beta_{11}, \beta_{22}, \beta_{33}$, β_{44} and β_{55} are the quadratic coefficients, $\in$ is the error term. For the regression analysis of the experimental data, design expert version 8.0.3.1 (Stat-Ease Inc., Minneapolis, MN, USA) software was used.

2.6.2. Statistical Data Analysis by Aartificial Neural Network.

A commercial ANN software, NeuralPower 2.5 (CPC-X software) was used, the learning algorithms used was QuickProp with multilayer normal feed forward (MNFF), three total layer numbers were used and the node number of input layer was five. Similarly, 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 using root mean square error (RMSE) approach. The higher coefficient of determination (R^2) was evaluated. The variable analysis also was conducted to study the effects of variables towards the L-phenylacetylcarbinol production using 3D curvature's surface plots.

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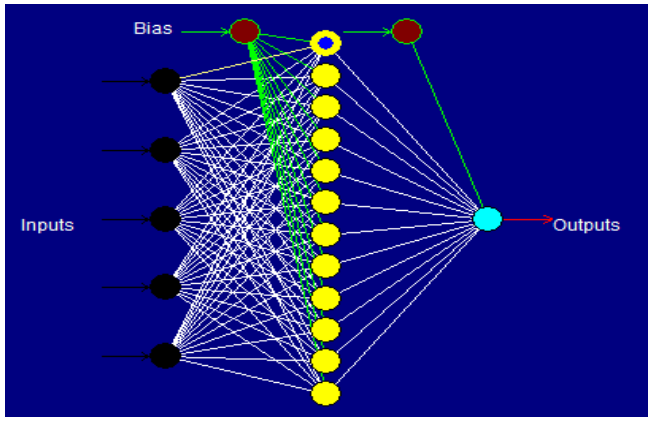

Fig-1. Structural network for a twelve transfer functions

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

Std. run	X_1	X_2	X_3	X_4	X_5		(mg/100 ml)	
						Actual L-PAC	RSM	ANN
1	-1	-1	-1	-1	-1	212.00	211.89	212
2	1	-1	-1	-1	-1	220.00	220.12	220
3	-1	1	-1	-1	-1	211.00	210.72	211
4	1	1	-1	-1	-1	210.00	210.33	210
5	-1	-1	1	-1	-1	209.00	209.04	209
6	1	-1	1	-1	-1	213.00	212.90	213
7	-1	1	1	-1	-1	211.00	210.74	211
8	1	1	1	-1	-1	206.00	205.98	206
9	-1	-1	-1	1	-1	205.00	205.22	205
10	1	-1	-1	1	-1	206.00	205.57	206
11	-1	1	-1	1	-1	205.00	205.42	205
12	1	1	-1	1	-1	197.00	197.16	197
13	-1	-1	1	1	-1	201.00	200.49	201
14	1	-1	1	1	-1	196.00	196.47	196
15	-1	1	1	1	-1	204.00	203.57	204
16	1	1	1	1	-1	191.00	190.93	191
17	-1	-1	-1	-1	1	332.00	332.23	332
18	1	-1	-1	-1	1	364.00	364.09	364
19	-1	1	-1	-1	1	368.00	367.94	368
20	1	1	-1	-1	1	391.00	391.17	391
21	-1	-1	1	-1	1	392.00	391.51	392
22	1	-1	1	-1	1	419.00	418.99	419
23	-1	1	1	-1	1	430.00	430.09	430
24	1	1	1	-1	1	449.00	448.95	449
25	-1	-1	-1	1	1	477.00	476.69	477
26	1	-1	-1	1	1	500.00	500.67	500
27	-1	1	-1	1	1	514.00	513.77	514
28	1	1	-1	1	1	529.00	529.13	529
29	-1	-1	1	1	1	534.00	534.09	534
30	1	-1	1	1	1	554.00	553.70	554

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31	-1	1	1	1	1	574.00	574.04	574
32	1	1	1	1	1	585.00	585.03	585
33	-2	0	0	0	0	345.00	345.61	345
34	2	0	0	0	0	369.00	368.46	369
35	0	-2	0	0	0	305.00	305.10	323
36	0	2	0	0	0	341.00	340.97	323
37	0	0	-2	0	0	277.00	276.49	308
38	0	0	2	0	0	339.00	339.58	308
39	0	0	0	-2	0	216.00	216.09	216
40	0	0	0	2	0	370.00	369.98	370
41	0	0	0	0	-2	52.00	52.14	52
42	0	0	0	0	2	664.00	663.92	664
43	0	0	0	0	0	386.00	386.51	386.5
44	0	0	0	0	0	387.00	386.51	386.5
45	0	0	0	0	0	386.00	386.51	386.5
46	0	0	0	0	0	387.00	386.51	386.5
47	0	0	0	0	0	386.00	386.51	386.5
48	0	0	0	0	0	387.00	386.51	386.5
49	0	0	0	0	0	386.00	386.51	386.5
50	0	0	0	0	0	387.00	386.51	386.5

3. DISCUSSION OF OBTAINED RESULTS

3.1. Results of RSM and its Discussion

Table 2 shows the coded factors considered in this study with L- Phenylacetylcarbinol concentration, 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 (Eq. (1)) and their arithmetical meaning.

Described in Table 3 is the results of test of significance for regression coefficient, with the large F-values and low equivalent p-values itemized in the table showed that all the model terms are noteworthy and this indicated very robust effects on the L-PAC concentration with $p< 0.05$. Nevertheless, the linear term X_5 with F-value of $3.61x10^6$ with p-value of <0.0001, was the most significant model term.

Table-3. Regression coefficient terms and their test of significance

Source	Sum of squares	df	Mean Square	F-value	p-value
X_1	999.64	1	999.64	5031.47	<0.0001
X_2	2463.02	1	2463.02	12397.09	<0.0001
X_3	7618.98	1	7618.98	38348.51	<0.0001
X_4	45333.77	1	45333.77	$2.282x\ 10^5$	<0.0001
X_5	$7.164x\ 10^5$	1	$7.164x\ 10^5$	$3.61x\ 10^6$	<0.0001
X_1X_2	148.78	1	148.78	748.86	<0.0001
X_1X_3	38.28	1	38.28	192.68	<0.0001
X_1X_4	124.03	1	124.03	624.28	<0.0001
X_1X_5	1116.28	1	1116.28	5618.56	<0.0001
X_2X_3	16.53	1	16.53	83.21	<0.0001
X_2X_4	3.78	1	3.78	19.03	<0.0001
X_2X_5	2719.53	1	2719.53	13688.18	<0.0001
X_3X_4	7.03	1	7.03	35.39	<0.0001

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X_3X_5	7719.03	1	7719.03	38852.10	<0.0001
X_4X_5	45677.53	1	45677.53	2.299x 10^5	<0.0001
X_1^2	1508.47	1	1508.47	7592.59	<0.0001
X_2^2	6996.22	1	6996.22	35213.98	<0.0001
X_3^2	10693.63	1	10693.63	53824.12	<0.0001
X_4^2	15172.48	1	15172.48	76367.46	<0.0001
X_5^2	1407.85	1	1407.85	7086.11	<0.0001

Table 4 showed the results of ANOVA for the second-order response surface model.

Table-4. ANOVA of regression equation

Source	Sum of Squares	df	Mean Square	F-value	p-value
Model	5.568 x 10^5	20	42838.92	2.156 x 10^5	<0.0001
Residual	5.76	29	0.20		
Lack of fit	3.76	22	0.17	0.60	0.8317
Pure error	2.00	7	0.29		
Cor total	8.568 x 10^5	49			

The model F-value (terms used to estimate effects) of 2.156 x10^5 with low p-value of <0.0001, inferred a high connotation for the regression model. The goodness of fit of the model was checked by the square of sample correlation coefficient (R^2). R^2 of 0.80 is acceptable for the good fit of a model [15]. The high value of R^2 (0.9750) obtained in this study indicated that the sample variation of 97.50% for the L-PAC production is attributed to the independent factors (cell mass incubation duration, concentration of acetaldehyde, concentration of benzaldehyde and β-cyclodextrin level). The value of the adjusted square of sample correlation coefficient (Adj. R^2) was 0.9742 and all p-values were less than 0.05, implying that the model proved its ~~suitable~~ suitability 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. Hence, the model could be used in theoretical prediction of the L- PAC production. The developed regression model equation describing the factors of cell weight (X_1), incubation duration(X_2),concentration of acetaldehyde (X_3), benzaldehyde (X_4) and β-cyclodextrin level (X_5) and their respective interactions is described in Eq. (2).

Where Y

Found in Table 5 is the coded coefficient values. The low values of error observed in the intercept and all the coefficient terms revealed that the data fits well with regression model with good prediction. The variance inflation factor (VIF) found in this study showed that the centre points are orthogonal to all other factors in the model.

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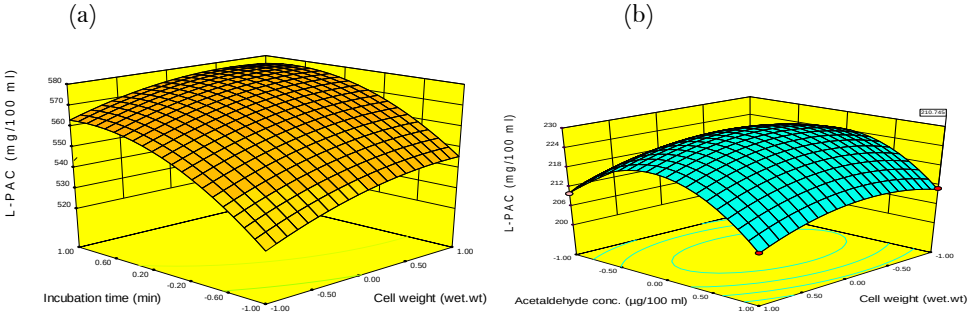
Table-5. Coefficients of regression and significance of second order response surface

Fact.	Coefficient estimate	df	Standard error	95% CI Low	95% CI high	VIF
Intercept	386.51	1	0.16	386.19	386.83	-
X_1	4.80	1	0.068	4.67	4.94	1.00
X_2	7.54	1	0.068	7.40	7.68	1.00
X_3	13.26	1	0.068	13.12	13.12	1.00
X_4	32.35	1	0.068	32.21	32.21	1.00
X_5	128.61	1	0.068	128.47	128.75	1.00
X_1X_2	-2.16	1	0.079	-2.32	-2.00	1.00
X_1X_3	-1.09	1	0.079	-1.25	-0.93	1.00
X_1X_4	-1.97	1	0.079	-2.13	-1.81	1.00
X_1X_5	5.91	1	0.079	5.75	6.07	1.00
X_2X_3	0.72	1	0.079	0.56	0.88	1.00
X_2X_4	0.34	1	0.079	0.18	0.50	1.00
X_2X_5	9.22	1	0.079	9.06	9.38	1.00
X_3X_4	-0.47	1	0.079	-0.63	-0.31	1.00
X_3X_5	15.53	1	0.079	15.37	15.69	1.00
X_4X_5	37.78	1	0.079	37.62	37.94	1.00
X_1^2	-5.21	1	0.060	-5.33	-5.09	1.05
X_2^2	-11.22	1	0.060	-11.34	-11.10	1.05
X_3^2	-13.87	1	0.060	-13.99	-13.75	1.05
X_4^2	-16.52	1	0.060	-16.65	-16.40	1.05
X_5^2	-5.03	1	0.060	-5.16	-4.91	1.05

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 bends' nature of 3D surfaces in Fig. 2a, b, e, f, and h suggested reciprocal interaction of cell mass with incubation duration, cell weight with concentration of acetaldehyde, incubation duration with concentration of acetaldehyde, incubation duration with concentration of benzaldehyde and concentration of acetaldehyde with concentration of benzaldehyde, respectively. On the other hand, the nature of curvatures' of 3D surfaces in Fig. 2c, d, g, i, j indicated moderate interactions of cell mass with concentration of benzaldehyde, cell mass with β-cyclodextrin level, incubation duration with β-cyclodextrin level, concentration of acetaldehyde with β-cyclodextrin level, and concentration of benzaldehyde with β-cyclodextrin level, respectively.



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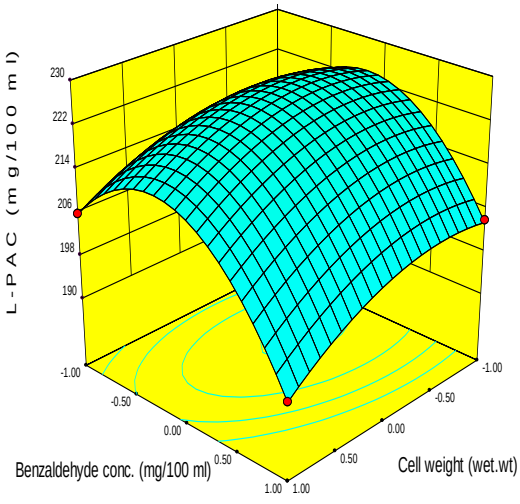

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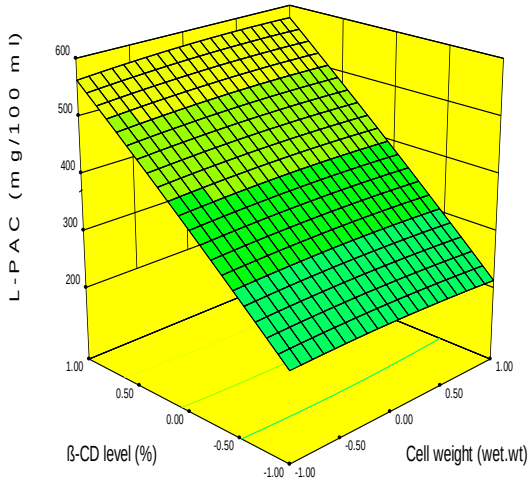
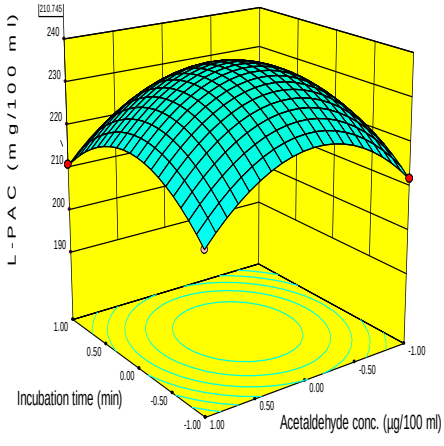


(e)



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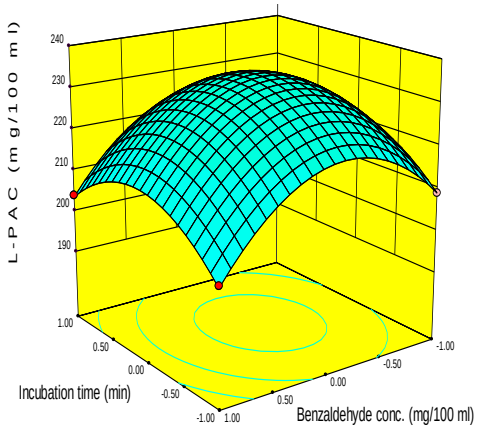

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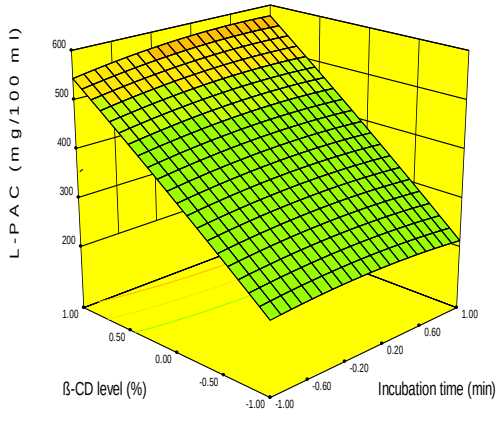

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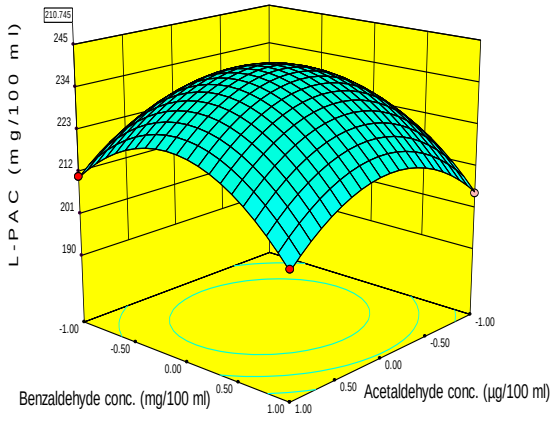

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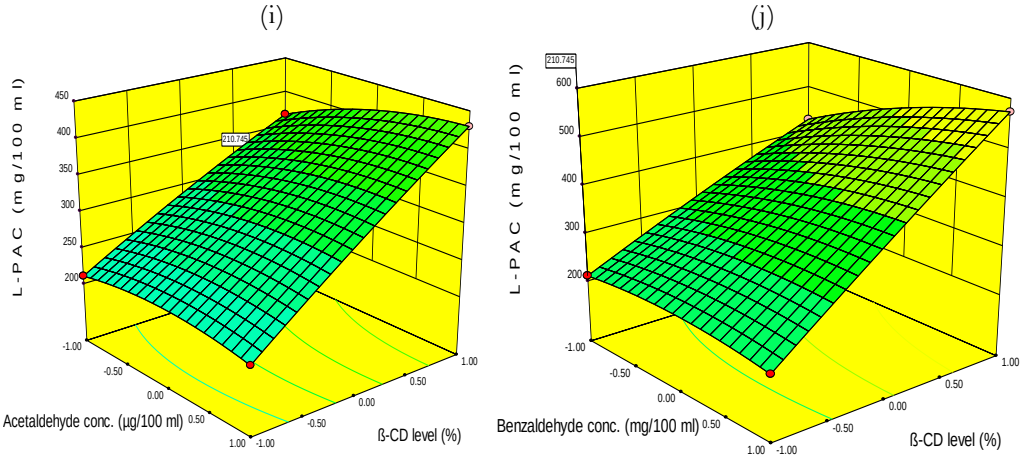

Fig-2. Three dimensional (3D) response surface plots (RSM)

The optimal values of the independent factors selected for the biotransformation of benzaldehyde to L- Phenylacetylcarbinol were obtained by solving the regression equation (Eqn. 2) using the Design-Expert software package. The optimal conditions for this process were statistically predicted as X_1 = 5.17 g (wet. wt.), X_2 = 74.82 (min), X_3 = 1594.05 (µl/100 ml), X_4 = 1300 (ml/100 ml) and X_5 = 3.20 %. The predicted L- Phenylacetylcarbinol concentration under the above set conditions was 586.938 (mg/100 ml). In order to verify the prediction of the model, the optimal conditions were applied to three independent replicates, and the average L-Phenylacetylcarbinol concentration obtained was 587.00 (mg/100 ml), which is well within the predicted value for the model equation.

3.2. Results of ANN and its Discussion

Depicts also in Table 2 was the observed concentrations as well as the difference obtained by ANN software. The effects of unexplained variability in the L- Phenylacetylcarbinol concentration 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). R^2 should be at least 0.80 for the good fit of a model [15]. In this case, the R^2 value of 0.9985 indicated that the sample variation of 99.85% for the L-PAC production is attributed to the independent factors (cell weight, incubation time, acetaldehyde concentration, benzaldehyde concentration and β-cyclodextrin level). The values of RMSE and the adjusted determination coefficient (Adj. R^2) were also evaluated to be 0.0450 and 0.997, 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 bends' nature of 3D surfaces in Fig. 3c, f, h, j, suggested mutual reciprocal interaction of cell weight with concentration of benzaldehyde, incubation duration with concentration of

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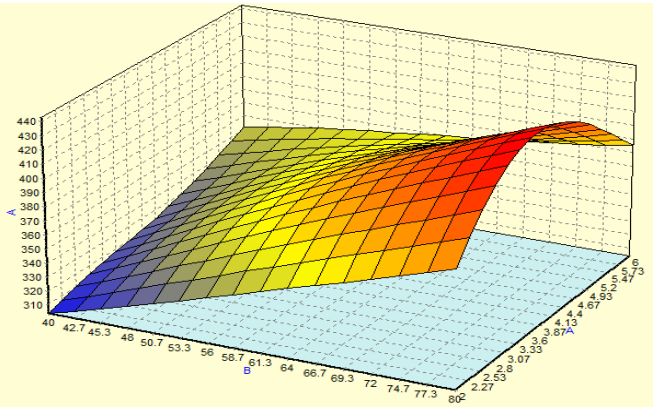
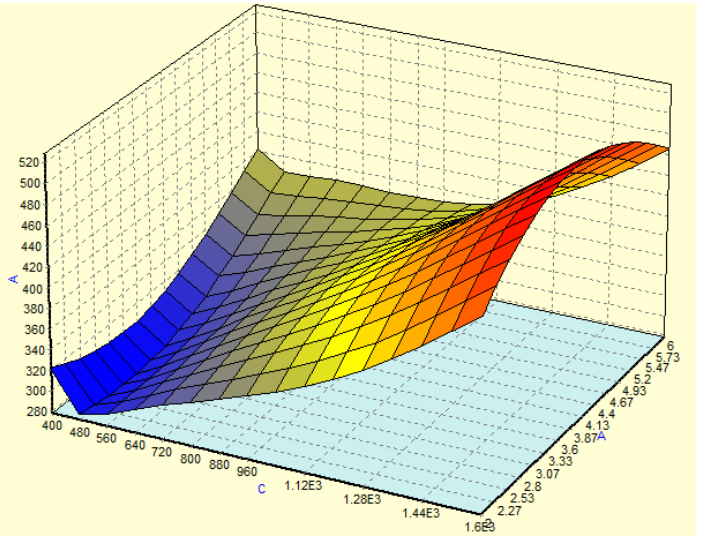
benzaldehyde, concentration of acetaldehyde with concentration of benzaldehyde, and concentration of benzaldehyde with β-cyclodextrin level, respectively. On the other hand, the nature of curvatures' of 3D surfaces in Fig. 3a, b, d, e, g, i, indicated moderate interactions of cell weight with incubation time, cell weight with concentration of acetaldehyde , cell mass with β-cyclodextrin level, incubation duration with concentration of acetaldehyde , incubation duration with β-cyclodextrin level, and concentration of acetaldehyde with β-cyclodextrin level, respectively. The optimal conditions for this process were statistically predicted as X_1 = 5.00 g (wet. wt.), X_2 = 70.12 (min), X_3 = 1582.00 (µl/100 ml), X_4 = 1300 (ml/100 ml) and X_5 = 3.00 %.



(a)

A(vertical) = L-PAC concentration (mg/100 ml), A(horizontal) = Cell mass (wet.wt), B(horizontal) = Incubation duration(min)



(b)

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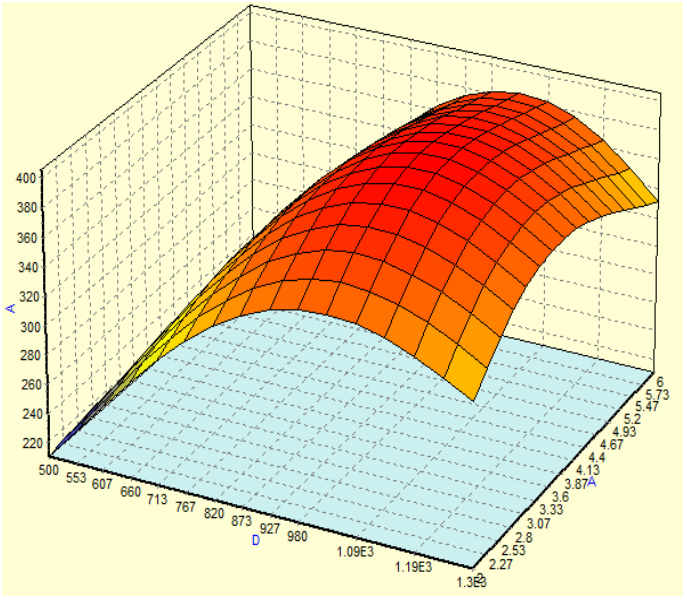
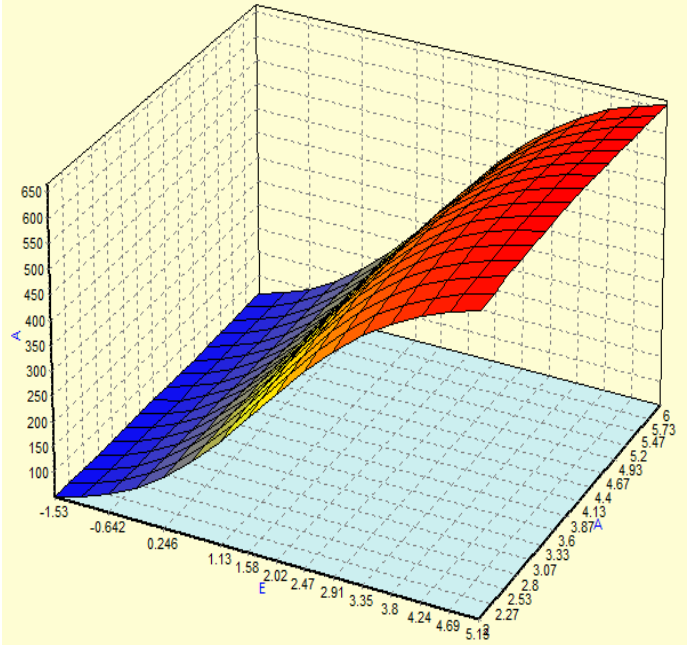
A(vertical) = L-PAC concentration (mg/100 ml), A(horizontal) = Cell mass (wet. wt.), C(horizontal) =concentration of acetaldehyde (μg/100 ml)



(c)

A(vertical) = L-PAC concentration (mg/100 ml), A(horizontal) = Cell mass (wet. wt.), D(horizontal) = Concentration of benzaldehyde (mg/100 ml)



(d)

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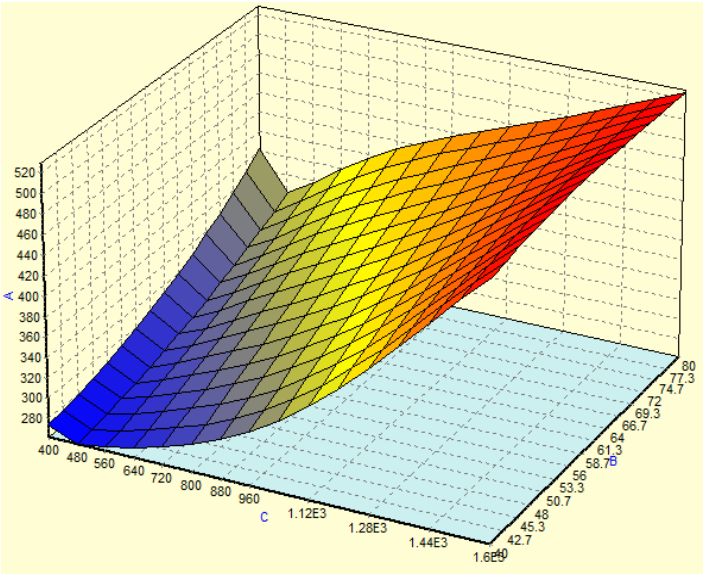
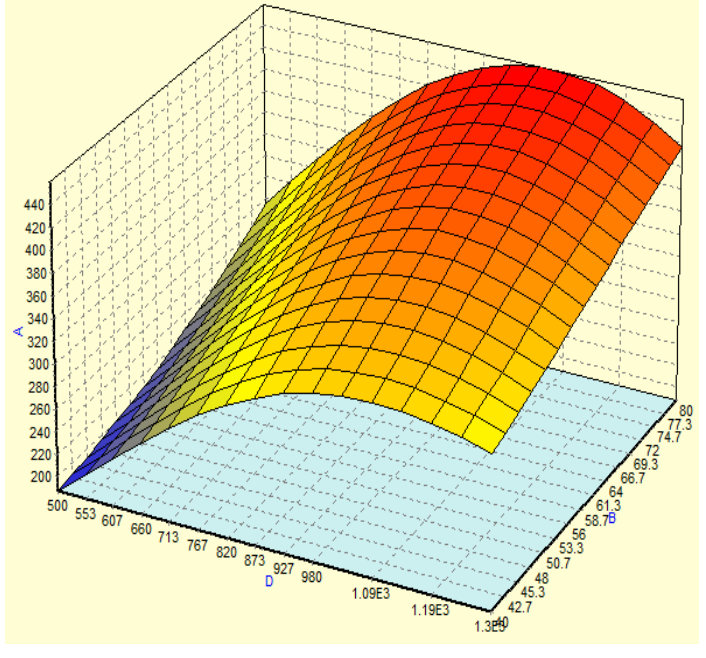
A(vertical) = BA concentration (mg/100 ml), A(horizontal) = Cell mass (wet. wt.), C(horizontal) = ß-CD level (%)



(e)

A(vertical) = L-PAC concentration (mg/100 ml), B(horizontal) = Incubation duration(min), C(horizontal) =concentration of acetaldehyde (µg/100 ml)



(f)

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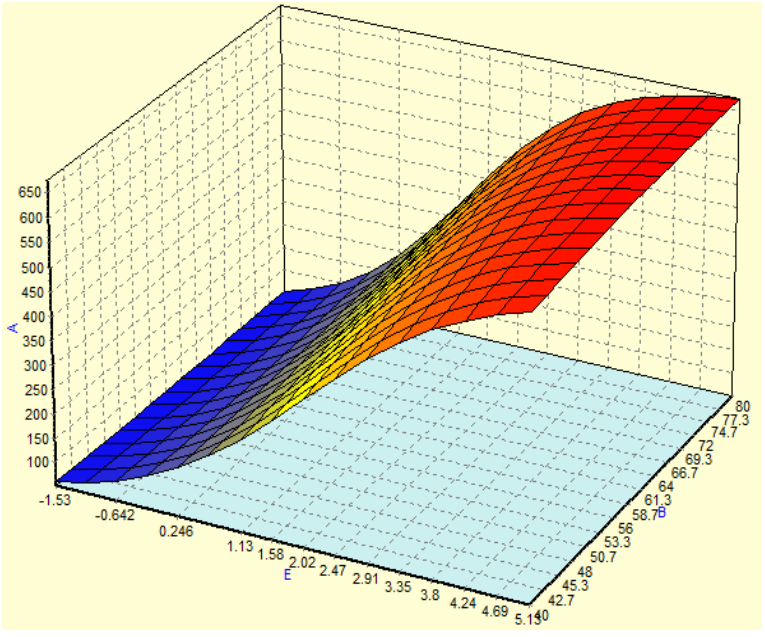
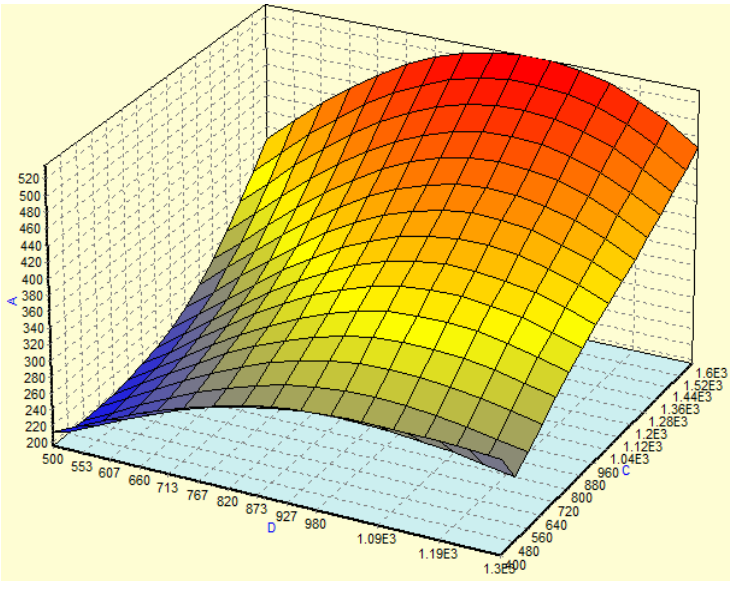
A(vertical) = L-PAC concentration (mg/100 ml), B(horizontal) = Incubation duration(min), D(horizontal) = Concentration of benzaldehyde (mg/100 ml)



(g)

A(vertical) = L-PAC concentration (mg/100 ml), B(horizontal) = Incubation duration(min), E(horizontal) = ß-CD level (%)



(h)

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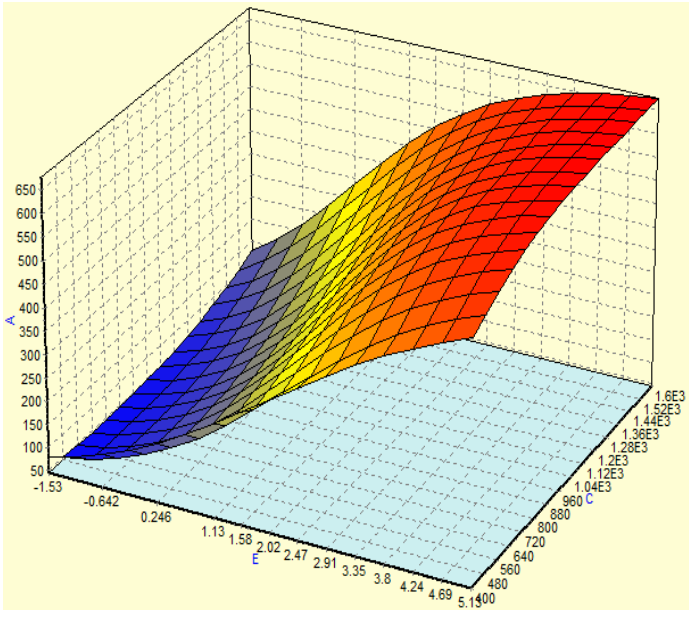
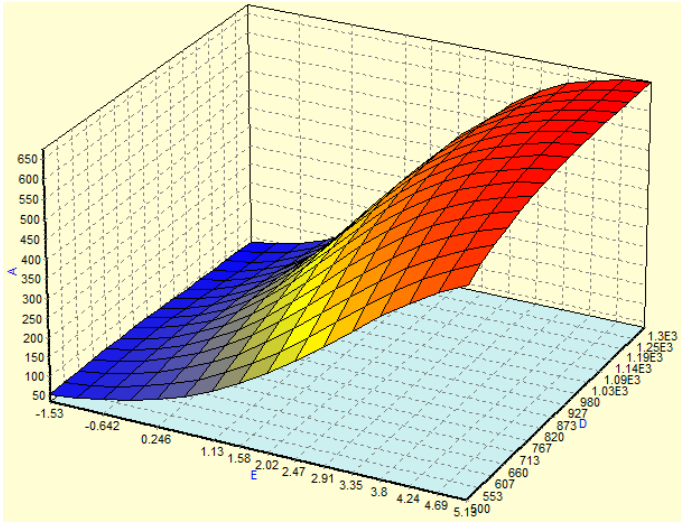
A(vertical) = L-PAC concentration (mg/100 ml), C(horizontal) =concentration of acetaldehyde (µg/100 ml), D(horizontal) = Concentration of benzaldehyde (mg/100 ml)



(i)

A(vertical) = L-PAC concentration (mg/100 ml), C(horizontal) =concentration of acetaldehyde (µg/100 ml), E(horizontal) = ß-CD level (%)



(j)

A(vertical) = L-PAC concentration (mg/100 ml), D(horizontal) = Concentration of benzaldehyde (mg/100 ml), E(horizontal) = ß-CD level (%)

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

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The predicted L- Phenylacetylcarbinol concentration under the above set conditions was 589.20 (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 L- Phenylacetylcarbinol concentration obtained was 590.24 (mg/100 ml), which is well within the predicted value for the model equation.

3.3. Assessment 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), average absolute deviation (AAD) and the average L-PAC concentration after validation. The comparative values RSME, R^2 and AAD are given in Table 6. The RMSE for the design matrix by RSM and ANN is 29% and 4.5%, the R^2 is 97.5% and 99.85%, and the AAD is 45% and 29.7%. The predicted (ANN) optimum emerged with the highest observed experimental L- Phenylacetylcarbinol production, with values above expectation (589.20 mg/100 ml). It should be noted that the experimenter did not have prior knowledge of models predictions. These observations showed that ANN derived models are more accurate in approximating the dynamics of L-Phenylacetylcarbinol biotransformation processes. The relatively low (586.938 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 nonlinear dynamics nature of biotransformation processes, being limited by its second- order quadratic polynomial function. Meanwhile, the excellent predictive (589.20 mg/100 ml) 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 nonlinear behaviour in the bioprocess dynamics.

Table-6. Assessment of RSM and ANN

Data	Values	
	RSM	**ANN**
AAD	0.450	0.297
RMSE	0.2900	0.0450
R^2	0.9750	0.9985
Adjusted R^2	0.9742	0.9970
Predicted (mg/100 ml)	586.938	589.20
L-PAC validated concentration (mg/100 ml)	587.00	590.24

4. CONCLUSSION

In this study, the effects of cell weight mass (wet. wt): X_1, incubation duration (min): X_2, concentration of acetaldehyde (mg/100 ml): X_3, concentration of benzaldehyde (mg/100 ml): X_4 and β-cyclodextrin level (%): X_5 were considered using RSM and ANN methods. The RMSE, R^2

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and AAD 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, it can be concluded that even though RSM is mostly used method for experimental optimization, the ANN methodology presented a better alternative.

5. ACKNOWLEDGEMENT

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