



## Effects of $\beta$ -Cyclodextrin on Phenyl Methanol Production and Its Optimization

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### Abstract

An Artificial Neural Network (ANN) was engaged to optimize the effect of  $\beta$ -Cyclodextrin on the production of Phenyl methanol (PM) from biotransformation of benzaldehyde by free cells of yeast. In developing ANN model, performance of ANN is heavily influenced by its network structure, five-level-five-factors design was applied, which generate 50 experimental runs. The inputs for the ANNs are cell weight (wet. wt):  $X_1$ , incubation time (min):  $X_2$ , Acetaldehyde conc. (mg/100 ml):  $X_3$ , benzaldehyde conc. (mg/100 ml):  $X_4$ , and  $\beta$ -level (%):  $X_5$ . The learning algorithms used was QP with MNFF, the transfer function was Tanh. Meanwhile, RMSE was determined to be 3.0739. The coefficient of determination  $R^2$  and the adj.  $R^2$  were found to be 0.99206 and 0.98419, respectively. It was observed that 900 (mg/100 ml) benzaldehyde with 1000 ( $\mu$ g/100 ml) acetaldehyde in the presence of 1.8%  $\beta$ -cyclodextrin gave the highest yield (351.5 mg/100 ml) of PM. Hence, it can be concluded that yeast (*Saccharomyces cerevisiae*) can tolerate higher levels of acetaldehyde and benzaldehyde due to the effects of  $\beta$ -cyclodextrin.

**Keywords:** Artificial neural network (ANN), Biotransformation, Yeast, Phenyl methanol, Optimization.

### 1.0 Introduction

Phenyl methanol (PM) is an aromatic alcohol with the molecular formula  $C_6H_5CH_2OH$ . It's a colorless liquid with a minor pleasant aromatic odour, partially soluble in water and completely miscible in alcohols and diethyl ether. Its polarity, low toxicity, and low vapor pressure make it usefulness a broadly one. Adepoju *et al.* (2013).

PM can be prepared by the hydrolysis of benzyl chloride using sodium hydroxide. It can also be produced by Grignard reaction. Meanwhile, PM can be used as general solvent in inks industries, paints industries, lacquers industries, and epoxy resin coatings industries (Furuta *et al.*, 1995; Adepoju *et al.*, 2013). It serves as an antecedent to a variety of esters, used in the soap, perfume, and flavour industries. It can be added to intravenous medication solutions as a preservative due to its bacteriostatic and antipruritic properties. It is also used as a photographic developer, used as a dielectric solvent for the dielectrophoretic reconfiguration of nanowires (Wissner-Gross, 2006; Adepoju *et al.*, 2013). It oxidized rapidly in healthy individuals to benzoic acid, conjugated with glycine in the liver, and excreted as hippuric acid. Though, high concentrations can result in toxic effects including respiratory failure, vasodilation, hypotension, paroxysms, and paralysis in newborns.

PM is produced naturally by many plants and is commonly found in fruits and teas, but can also be found in a variety of essential oils including jasmine, hyacinth, and ylang-ylang. It is one of the chemical compounds found in castoreum. It is also a bi-product in biotransformation of benzylaldehyde to Phenylacetylcarbinol (L-PAC) using free cells. Meanwhile, almost all the literature concerning the synthesis of PM by fermenting yeast deals with yield optimization by free cells (Agrawal *et al.*, 1986; Cardillo *et al.*, 1991; Zeeman *et al.*, 1992; Adepoju *et al.*, 2013).

The formation of bi-production (L-PAC, PAC-diol and residual benzene) along the production of PM from benzaldehyde under normal fermentative conditions using yeast shows that the assessable conversion has never been achieved (Smith and Hendlin, 1953; Gupta *et al.*, 1979; Netraval and Vojtisek, 1982; Agrawal and Basu, 1989; Adepoju *et al.*, 2013). Due to the yeast toxic and inhibitory effects of substrate and products, it cannot be used for multiple batches, except when accompanied with the use of cyclodextrin which always decreased the toxicity of benzaldehyde for bioconversion (Coughlin *et al.*, 1991; Mahmoud *et al.*, 1990; Adepoju *et al.*, 2013).

In this work, an Artificial Neural Network (ANN) was engaged to optimize qualitative conversion of benzaldehyde to phenyl methanol (PM) by free cell of yeast (*Saccharomyces cerevisiae*) and the effect of  $\beta$ -Cyclodextrin was appraised.

### 2.0 Material and Methods

#### 2.1 Materials

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

#### 2.2 Methods

##### 2.2.1 Microorganisms

Yeast used in this study was isolated locally. The culture was consistently maintained on a medium containing 0.4% dextrose, 1% yeast extract, 1% malt extract, and 2% agar at pH 7.2 (Agrawal *et al.*, 1986; Adepoju *et al.*, 2013).

### 2.2.2 The growth medium

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

### 2.2.3 Culture growth

1.0 ml suspension of cells of the isolate *Saccharomyces cerevisiae* containing  $10^6$  cells was inoculated into 9 ml of growth medium and incubated on a rotary shaker at  $30 \pm 2^\circ\text{C}$  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  $15^\circ\text{C}$ . The biomass obtained was washed with water, centrifuged and was used for biotransformation studies.

### 2.2.4 Biotransformation of benzaldehyde to L-PAC

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

### 2.2.5 Effect of $\beta$ -cyclodextrin addition on biotransformation of benzaldehyde

Effect of various levels of  $\beta$ -cyclodextrin was studied at benzaldehyde and acetaldehyde levels ranging from 500 mg to 1600 mg/100 ml and 400  $\mu\text{l}$  to 1300  $\mu\text{l}$ /100 ml, respectively. The reaction was allowed to take place for 3 h at  $30 \pm 2^\circ\text{C}$  and 240 rpm. To study the effect of  $\beta$ -CD level, concentration of  $\beta$ -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 software (Table 1).

## 2.3 Analysis of biotransformation products

After biotransformation, the medium was centrifuged at 10,000 rpm for 15 min. The supernatant were extracted three times with equal volumes of diethyl ether. The combined 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

The conditions used for GC analysis were as follows- GC model 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  $250^\circ\text{C}$ . Column programming was as follows:  $75^\circ\text{C}$  for 3 min, then  $10^\circ\text{C}/1\text{ min}$  up to  $250^\circ\text{C}$  and holding time was for 5 min. Retention times of L-PAC was 17 min. The concentration of the compound was determined using peak area method (Shukla and Kulkarni, 1999, Adepoju *et al.*, 2013). The experiment was replicated in triplicate until it was found to be reproducible within  $\pm 3$  percent limits.

## 2.5 Experimental design

In developing ANN model, performance of ANN is heavily influenced by its network structure; therefore, the learning algorithms used 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 mean square error (MSE) approach. The higher coefficient  $R^2$  was determined; the variable analysis also was conducted to study the effects of variables towards the PM production using 3D curvatures' surface plots. A hybrid ANN model was used in conducting process optimization.

Table 1 show the independent factors 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  $\beta$ -CD level (%):  $X_5$ , and their five levels for ANNs design using response surface methodology (RSM). Table 2 depicts the PM yields, the observed values and the difference. The effects of unexplained variability in the PM yield response due to extraneous factors were minimized by randomizing the order of experiments.

**Table 1: Factors and their Levels for ANN Design using RSM**

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. ( $\mu\text{g}/100\text{ ml}$ )	$X_3$	400	700	1000	1300	1600
Benzaldehyde conc. (mg/100 ml)	$X_4$	500	700	900	1100	1300
$\beta$ -CD level (%)	$X_5$	0.4	0.8	1.2	1.6	3.2

**Table 2: ANN Design for Biotransformation of Benzaldehyde to L-PAC and the by products with Five Independent Variables using actual values**

Std. run	X <sub>1</sub>	X <sub>2</sub>	X <sub>3</sub>	X <sub>4</sub>	X <sub>5</sub>	PM (mg/100 ml)	Observed value (mg/100 ml)	Difference
1	2.00	40.00	400.00	500.00	0.40	259	259	1.7053E-13
2	6.00	40.00	400.00	500.00	0.40	276	276	1.7053E-13
3	2.00	80.00	400.00	500.00	0.40	300	300	2.8422E-13
4	6.00	80.00	400.00	500.00	0.40	298	298	3.979E-13
5	2.00	40.00	1600.00	500.00	0.40	309	309	3.4106E-13
6	6.00	40.00	1600.00	500.00	0.40	311	311	3.4106E-13
7	2.00	80.00	1600.00	500.00	0.40	338	338	1.1369E-13
8	6.00	80.00	1600.00	500.00	0.40	321	321	3.4106E-13
9	2.00	40.00	400.00	1300.00	0.40	273	273	1.7053E-13
10	6.00	40.00	400.00	1300.00	0.40	294	294	3.4106E-13
11	2.00	80.00	400.00	1300.00	0.40	304	304	5.6843E-14
12	6.00	80.00	400.00	1300.00	0.40	305	305	4.5475E-13
13	2.00	40.00	1600.00	1300.00	0.40	313	313	3.979E-13
14	6.00	40.00	1600.00	1300.00	0.40	319	319	2.2737E-13
15	2.00	80.00	1600.00	1300.00	0.40	331	331	3.4106E-13
16	6.00	80.00	1600.00	1300.00	0.40	317	317	2.2737E-13
17	2.00	40.00	400.00	500.00	3.20	305	305	1.7053E-13
18	6.00	40.00	400.00	500.00	3.20	321	321	3.4106E-13
19	2.00	80.00	400.00	500.00	3.20	332	332	2.8422E-13
20	6.00	80.00	400.00	500.00	3.20	327	327	2.8422E-13
21	2.00	40.00	1600.00	500.00	3.20	300	300	2.2737E-13
22	6.00	40.00	1600.00	500.00	3.20	299	299	2.2737E-13
23	2.00	80.00	1600.00	500.00	3.20	314	314	2.8422E-13
24	6.00	80.00	1600.00	500.00	3.20	294	294	3.4106E-13
25	2.00	40.00	400.00	1300.00	3.20	282	282	2.2737E-13
26	6.00	40.00	400.00	1300.00	3.20	300	300	3.4106E-13
27	2.00	80.00	400.00	1300.00	3.20	297	297	4.5475E-13
28	6.00	80.00	400.00	1300.00	3.20	296	296	1.1369E-13
29	2.00	40.00	1600.00	1300.00	3.20	266	266	1.1369E-13
30	6.00	40.00	1600.00	1300.00	3.20	268	268	2.8422E-13
31	2.00	80.00	1600.00	1300.00	3.20	269	269	5.6843E-14
32	6.00	80.00	1600.00	1300.00	3.20	251	251	3.979E-13
33	2.00	60.00	1000.00	900.00	1.80	300	300	3.4106E-13
34	6.00	60.00	1000.00	900.00	1.80	302	302	2.8422E-13
35	4.00	40.00	1000.00	900.00	1.80	337	351.5	14.5
36	4.00	80.00	1000.00	900.00	1.80	366	351.5	14.5
37	4.00	60.00	400.00	900.00	1.80	297	301.5	4.5
38	4.00	60.00	1600.00	900.00	1.80	306	301.5	4.5
39	4.00	60.00	1000.00	500.00	1.80	320	320	3.979E-13
40	4.00	60.00	1000.00	1300.00	1.80	288	288	2.8422E-13
41	4.00	60.00	1000.00	900.00	0.40	266	266	1.7053E-13
42	4.00	60.00	1000.00	900.00	1.80	245	245	3.6948E-13
43	4.00	60.00	1000.00	900.00	1.80	277	277.5	0.5
44	4.00	60.00	1000.00	900.00	1.80	278	277.5	0.5
45	4.00	60.00	1000.00	900.00	1.80	277	277.5	0.5
46	4.00	60.00	1000.00	900.00	1.80	278	277.5	0.5
47	4.00	60.00	1000.00	900.00	1.80	277	277.5	0.5
48	4.00	60.00	1000.00	900.00	1.80	278	277.5	0.5
49	4.00	60.00	1000.00	900.00	1.80	277	277.5	0.5
50	4.00	60.00	1000.00	900.00	1.80	278	277.5	0.5

### 2.5.1 Statistical Data Analysis

ANN structure was used for modelling the PM production. The optimum ANN structure was determined using mean square error (MSE) approach. The higher coefficient  $R^2$  was determined; the variable analysis also was conducted to study the effects of variables towards the PM yield using relative importance and 3D curvatures' surface plots. A hybrid ANN model was used in conducting process optimization. The difference between the experimental and observed values was also used to proof the validity of ANN for the optimization of PM production.

### 3.0 Results and Discussion

Table 2 depicts the actual factors considered in this study with experimental PM yields, the observed yields as well as the difference obtained by ANN software. The effects of unexplained variability in the L-PAC yield response due to

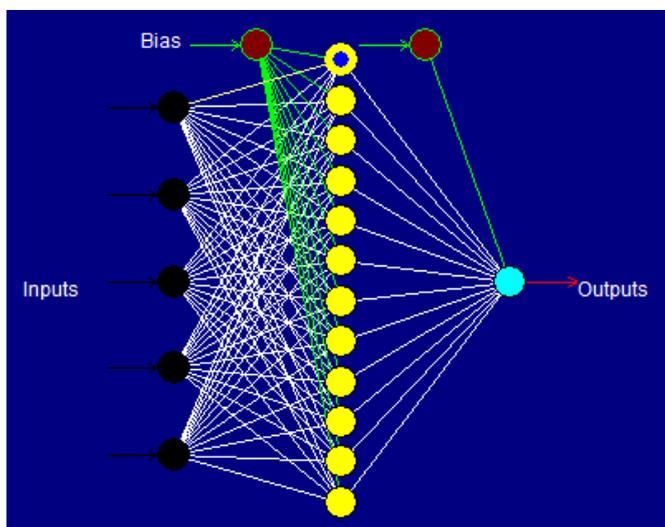
extraneous factors were minimized by randomizing the order of experiments. Table 3 shows the parameters of the best network that described the results of the normal data type for ANN. Considering the large QP-values (the number of repetition) and low corresponding RMSE-values (root mean squared error) which was used to compare the predicted values of L-PAC yield obtained from the model with experimental data, shows that all the model terms are significant and have very strong effects on the L-PAC yield. 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 (Guan and Yao, 2008). In this case, the  $R^2$  value of 0.99206 indicated that the sample variation of 99.206% for the PM production is attributed to the independent factors (cell weight, incubation time, acetaldehyde concentration, benzaldehyde concentration and  $\beta$ -CD level). The value of the adjusted determination coefficient (Adj.  $R^2$ ) was also evaluated to be 0.98419.

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

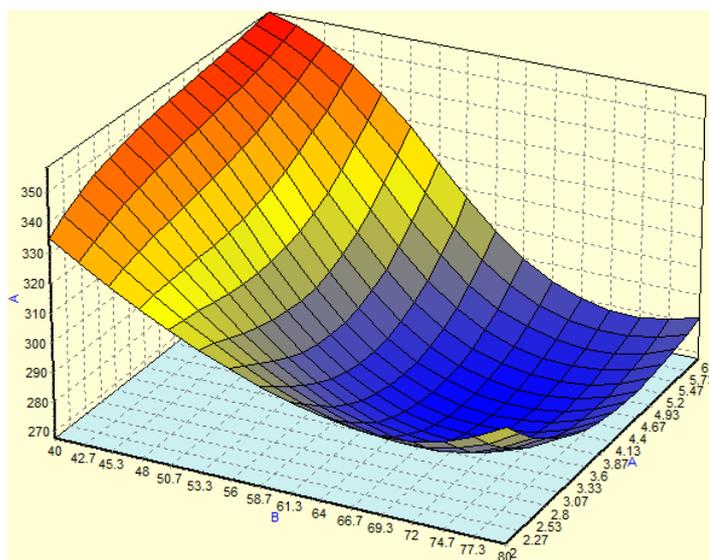
Fig. 2. The curvatures' nature of 3D surfaces in Fig. 2a, c, d, e, f, g, j, suggested mutual reciprocal interaction of cell weight with incubation time, cell weight with benzaldehyde concentration, cell weight with  $\beta$ -CD level, incubation time with acetaldehyde concentration, incubation time with benzaldehyde concentration, incubation time with  $\beta$ -CD level, and benzaldehyde concentration with  $\beta$ -CD level, respectively. On the other hand, the nature of curvatures' of 3D surfaces in Fig. 2b, h, i, indicated moderate interactions of cell weight with acetaldehyde concentration, acetaldehyde concentration with benzaldehyde concentration and acetaldehyde concentration with  $\beta$ -CD level, respectively.

**Table 3: Parameters of the Best Network for PM Describing the Results of the Normal Data Type For ANNs**

Data	Values
Iteration (QP)	608100
RMSE	3.0739
Average R	0.99206
Average DC	0.98419

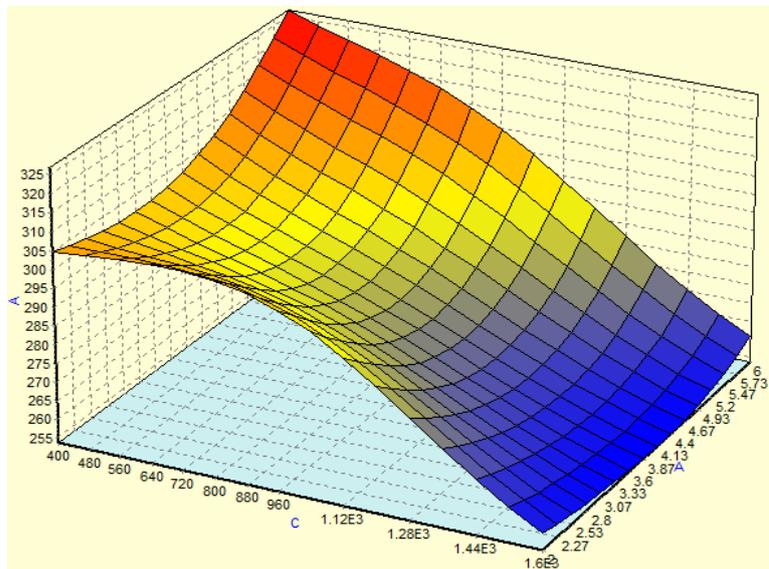


**Fig. 1: Network Structure with Twelve Transfer Functions**



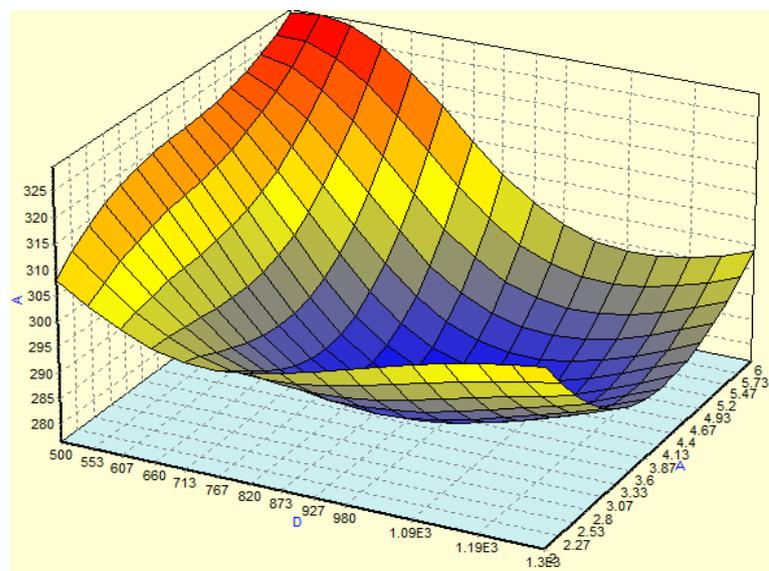
(a)

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



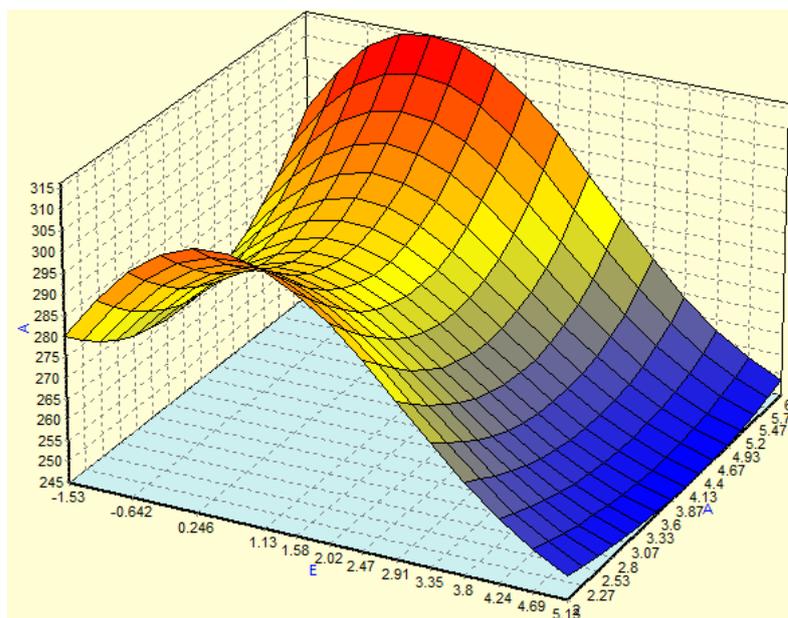
(b)

A(vertical) = BA yield (mg/100 ml), A(horizontal) = Cell weight g(wet.wt), C(horizontal) = Acetaldehyde conc. ( $\mu\text{g}/100\text{ ml}$ )



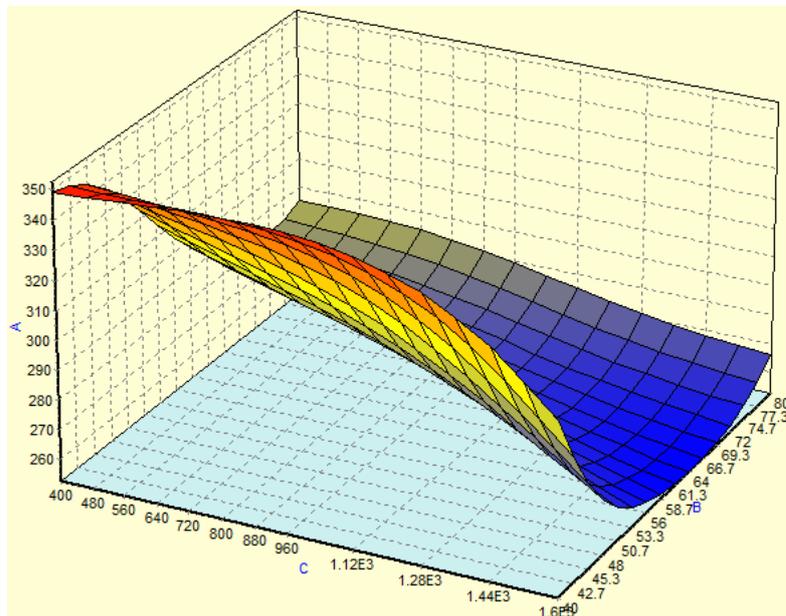
(c)

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



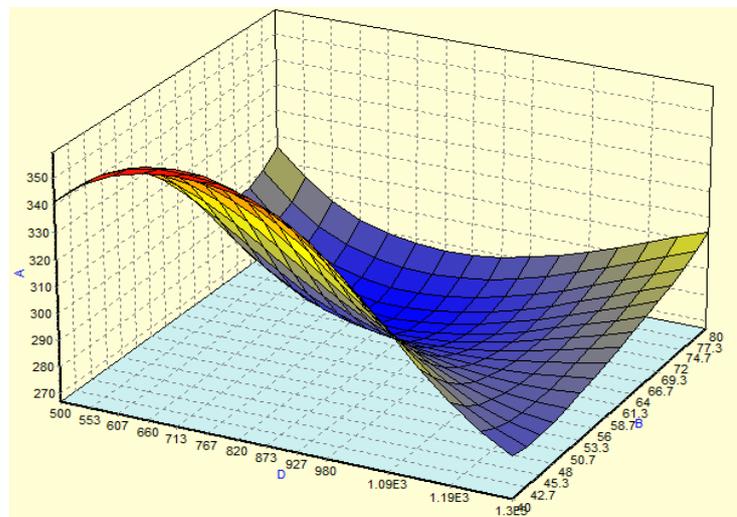
(d)

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



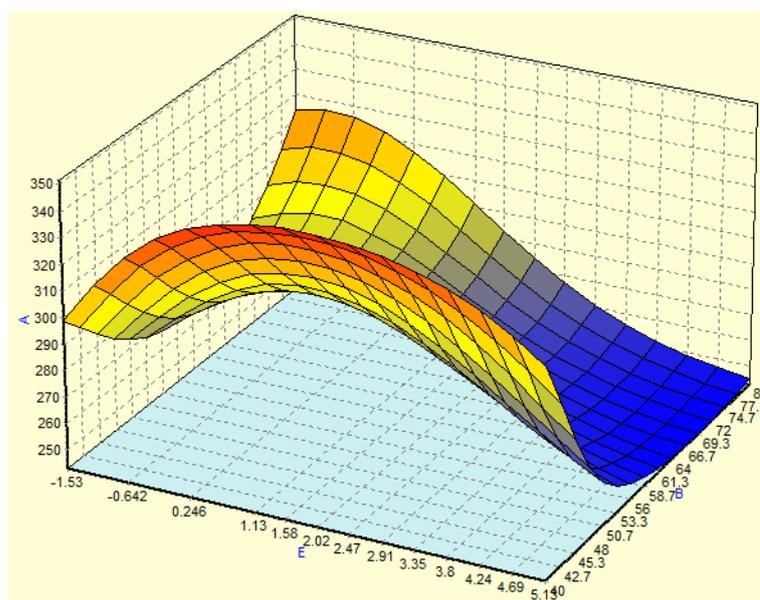
(e)

A(vertical) = BA yield (mg/100 ml), B(horizontal) = Incubation time (min), C(horizontal) = Acetaldehyde conc. ( $\mu\text{g}/100$  ml)



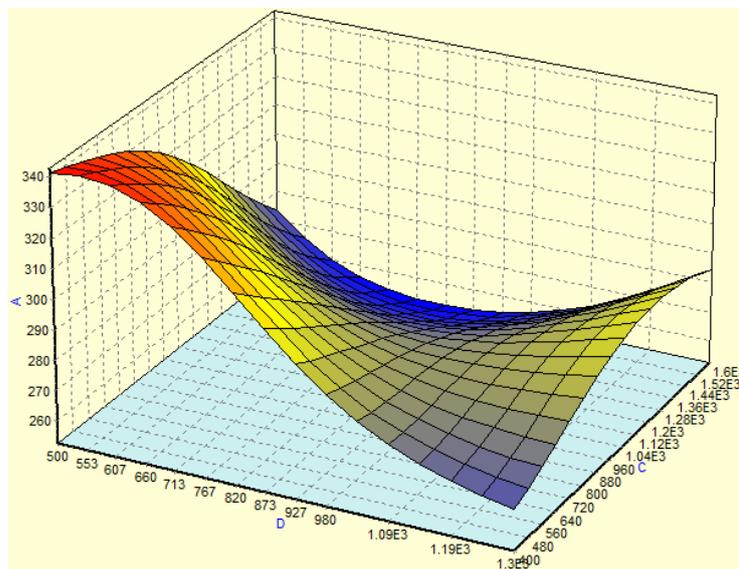
(f)

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



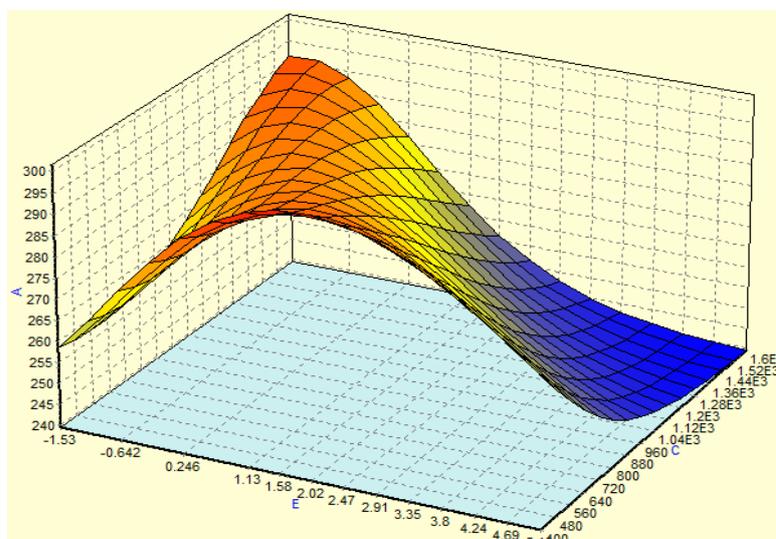
(g)

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



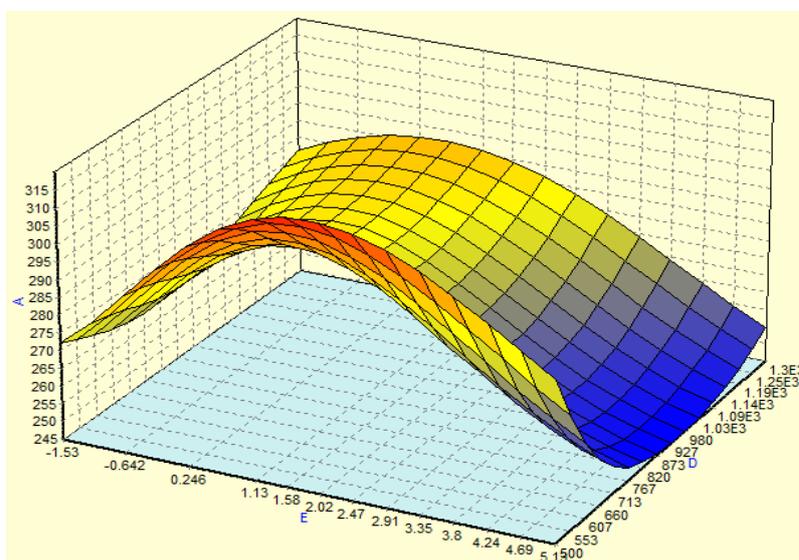
(h)

A(vertical) = BA yield (mg/100 ml), C(horizontal) = Acetaldehyde conc. ( $\mu\text{g}/100\text{ ml}$ ), D(horizontal) = Benzaldehyde conc. (mg/100 ml)



(i)

A(vertical) = BA yield (mg/100 ml), C(horizontal) = Acetaldehyde conc. ( $\mu\text{g}/100\text{ ml}$ ), E(horizontal) =  $\beta$ -CD level (%)



(j)

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

**Fig. 2: (a-j): 3-D curvatures' plots**

## Conclusions

The results obtained in this study indicate that ANN is a good optimization tools for PM production. The Root Mean Square Error (RMSE) obtained was 3.0739. The coefficient of determination  $R^2$  and the adj.  $R^2$  were found to be 0.99206 and 0.98419, respectively. It was observed that 1000 (mg/100 ml) benzaldehyde with 900 ( $\mu\text{g}/100\text{ ml}$ ) acetaldehyde in the presence of 1.8%  $\beta$ -cyclodextrin gave the highest yield of PM. Hence, the organism can tolerate higher levels of acetaldehyde and benzaldehyde due to effects of  $\beta$ -cyclodextrin.

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