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A N I N T E R N A T I O N A L J O U R N A L

Growth Characteristics Modeling of *Bifidobacterium bifidum* Using RSM and ANN

Ganga S. Meena, Suneel Gupta, Gautam C. Majumdar and Rintu Banerjee*

Microbial Biotechnology and Downstream Processing Laboratory; Department of Agricultural and Food Engineering; Indian Institute of Technology; Kharagpur-721 302 - India

ABSTRACT

The aim of this work was to optimize the biomass production by Bifidobacterium bifidum *255 using the response surface methodology (RSM) and artificial neural network (ANN) both coupled with GA. To develop the empirical model for the yield of probiotic bacteria, additional carbon and nitrogen content, inoculum size, age, temperature and pH were selected as the parameters. Models were developed using ¼ fractional factorial design (FFD) of the experiments with the selected parameters. The normalized percentage mean squared error obtained from the ANN and RSM models were 0.05 and 0.1%, respectively. Regression coefficient (R^2) of the ANN model showed higher prediction accuracy compared to that of the RSM model. The empirical yield model (for both ANN and RSM) obtained were utilized as the objective functions to be maximized with the help of genetic algorithm. The optimal conditions for the maximal biomass yield were 37.4 °C, pH 7.09, inoculum volume 1.97 ml, inoculum age 58.58 h, carbon content 41.74% (w/v), and nitrogen content 46.23% (w/v). The work reported is a novel concept of combining the statistical modeling and evolutionary optimization for an improved yield of cell mass of* B. bifidum *255.*

Key words: Probiotics, response surface methodology (RSM), FFD, artificial neural network (ANN), genetic algorithms (GA)

INTRODUCTION

Bifidobacterium is the most prominent member of plethora class of bacterial species with probiotic properties. The popularity of this group of bacteria is based on the millennia of use in the food and feed that are used in the probiotic dairy drinks and yoghurts since long (Sanders, 1999). At present, in India, the production of probiotics is reported to grow annually about 22.6 % until 2015 and the market of the probiotics is ~20.6 million rupees (€320,000). The market demand indicates that it is economically viable product. The probiotics have immense application in the food/healthcare sector. There are plenty of industries venturing into the production and selling of the probiotics sachets to meet the increasing demand. Most common bacteria targeted by the industries for the probiotic sachet preparation includes *Bifidiobacterium*. Microbial colonization of the human intestine starts immediately after the birth (Gibson and Roberfroid, 1995). The predominant bacteria at the infancy stage are *Bifidobacteria* which colonize within the first 4-7 days of birth with the numbers ranging from 10^9-10^{10} CFU/g of faeces in breast-fed infants (Gismondo et al., 1999).

*Author for correspondence: rb@iitkgp.ac.in

Bifidobacterium sp. is one of the major microorganisms in the gastrointestinal tract flora of the children and adults. These bacteria have a strong stimulatory effect for the normal development of microbiota and maturation of gut associated lymphoid tissue (Schezenmeir and De Vrese, 2001). Probiotic bacteria such as *Bifidobacterium* and *Lactobacillus* sp. in the gastrointestinal tract can play an important role in promoting the human health (Savage, 1977; Mitsuoka, 1990). These microorganisms can contribute to digestion, immune stimulation and inhibition of the pathogens such as *Bacteroides, Escherichia, Clostriduim* and *Proteus* which are potentially harmful bacteria found in the gastrointestinal tract (Ziemer and Gibson, 1998).

The primary mechanism for probiotic action is known as competitive colonization or competitive suppression. It is best described as the proliferation of the probiotic bacteria in the human intestine, leaving little space for the growth of any pathogens (Ballongue, 1992; Biavati et al., 2000). To develop the growth model of probiotic bacteria through the traditional method, i.e. one variable at-a-time is time consuming and interactions of different variables can also affect the yield. Unlike the conventional optimization, the statistical optimization methods can take into account the interactions of the variables in generating the process response. Process optimization through the statistical method is a technique in which changes or adjustments are made in a process to get better results (Myers and Montgomery, 2002). There are several techniques for process optimization, i.e., Response Surface Methodology (RSM), Artificial Neural Networks (ANN), Genetic Algorithms (GA), etc. In these engineering applications, a response of interest is usually influenced by several variables and the objective of the engineering applications is to find the variables that can optimize the response. RSM is a tool on that basis we find the optimal process parameters that produce a maximum or minimum value of the response and represent the direct and interactive effects of the process parameters through two and three-dimensional plots (Gangadharan et al., 2008). Artificial neural networks are computational models of nervous systems. Natural organisms, however, do not possess only nervous systems but also genetic information stored in the nucleus of their cells (genotype). The nervous system is part of the phenotype which is derived from this genotype through the process of development (Rajasekaran and Vijaylakshmi, 2004). Using the method of neural networks (NN), the relationship between a set of independent variables *X* and the dependent variables *Y* can be obtained. From the given pairs of input *X* and output *Y* data, neural network directly learns, and then develops a relationship between them but does not yield any mathematical equation relating the variables. After the learning, this network is able to predict the correct output from an input data set that has not been previously used during the learning. Genetic algorithms (GA) are a tool by which the optimization problems can be accurately solved within a limited use of computer time (Das, 2005). The objective of this work was to optimize and improve the yield of probiotic bacteria, *Bifidobacterium bifidum by* optimizing the growth parameters such as temperature, pH, inoculum volume, inoculum age and additional effect of different carbon and nitrogen sources with the help of Response Surface Methodology, Artificial Neural Network and Genetic Algorithms.

MATERIALS AND METHODS

Organism and growth condition

Pure culture of *Bifidobacterium bifidum* 255 was obtained from the National Collection of Dairy Cultures (NCDC) Karnal, Haryana (India). The culture was grown in a modified MRS media containing 1% (w/v) sodium thiosulphate at 30°C under anaerobic condition. Biomass growth was determined by measuring the optical density (OD) at 600 nm.

Experimental design

Selection of initial parameters

For the selection of initial parameters, 'one variable at a time method' was used. The different variables *viz.* temperature, pH, volume of inoculum, age of inoculum and additional carbon and nitrogen sources were selected for growth of *B. bifidum*.

Empirical model development

To find out the effect of different growth parameters on the predicted value of the bacterial growth, *Yp* was obtained by conducting the experiments on different combination of independent variables (growth parameters), which was obtained from a standard experimental design. During the experiments, the 'response' or values

of 'dependent variables' obtained from each of the combinations of independent variables was measured. A mathematical relationship between the independent and dependent variables was developed. This relationship was called 'model'. Using this model, the predicted values of responses were found out within the domain of limiting values of independent variables. For the different growth parameters, a polynomial model was developed between the growth and growth parameters to find out the following relationship between the coded values x_1, x_2, x_3, x_4, x_5 and x_6 of independent variables and dependent variable Yp as shown below

$$Yp=b_o+b_1x_1+b_2x_2+b_3x_3+b_4x_4+b_5x_5+b_6x_6+b_7x_1^2+b_8x_2^2+b_9x_3^2+b_{10}x_4^2+b_{11}x_5^2+b_{12}x_6^2+b_{13}x_1x_2+b_{14}x_1x_3+b_{15}x_1x_4+b_{16}x_1x_5+b_{17}x_1x_6+b_{18}x_2x_3+b_{19}x_2x_4+b_{20}x_2x_5+b_{21}x_2x_6+b_{22}x_3x_4+b_{23}x_3x_5+b_{24}x_3x_6+b_{25}x_4x_5+b_{26}x_4x_6+b_{27}x_5x_6 \quad \text{(Eq. 1)}$$

Where b_o, b_1, b_2……etc. are the regression constants.

Experimental modeling

Fractional factorial design

Using two levels (+1 and -1) factorial design, two values of l and s for two sacrificing interactions were l_1, s_1, l_2 and s_2. With the help of factorial design, s values were identified as (s_1= 0, s_2 =0), (s_1= 0, s_2 =1), (s_1= 1, s_2 =0), and (s_1= 1, s_2 =1). In this study, all the experiments were conducted according to s_1= 0 and s_2 =0 design.

Optimization

Neural Network modeling

ANN chosen was a radial basis function network with supervised learning. The model was based on feed forward back propagation training method. In this process, the network computed the error between the desired output (predicted) and the actual (experimental) output. It trained the network to make adjustments to minimize the error and back propagate the same.

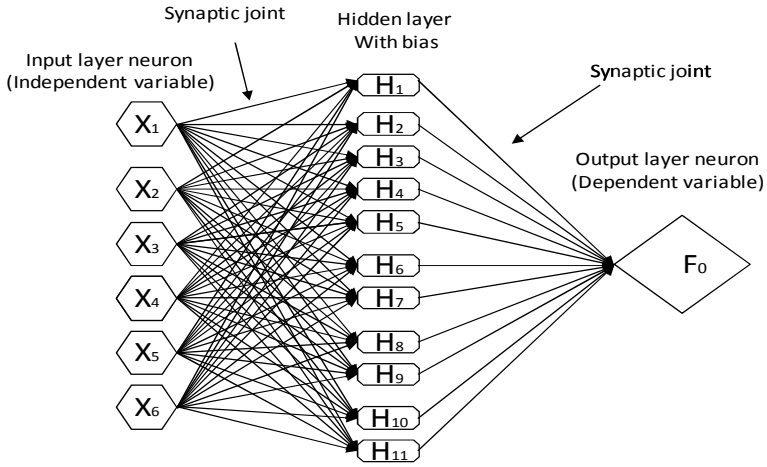

Figure 1 - Basic structure of a feed forward back propagation neural network.

Genetic Algorithms

In this optimization study, GA was applied to the developed ANN based model as shown in the Fig 2. The prime objective of this study was to maximize the biomass yield of gap *B. bifidum* by monitoring the growth parameters such as temperature, pH, inoculum volume, inoculum age, carbon % and nitrogen %. It was posed as the minimization of problem associated with the optimization studies. Genetic optimization continued till the termination condition i.e. maximum biomass yield was obtained.

Software used

For proper execution of ANN and GA, MATLAB 7.0 was used to develop the empirical model.

RESULTS AND DISCUSSION

Selection of initial parameters

Fig 3 (A-F) shows the effect of temperature, initial pH, initial inoculum volume, initial incubation period, supplementation of additional carbon and nitrogen sources on the growth of the bacterial culture. All these parameters, their variation and optimum values are given in Table 1.

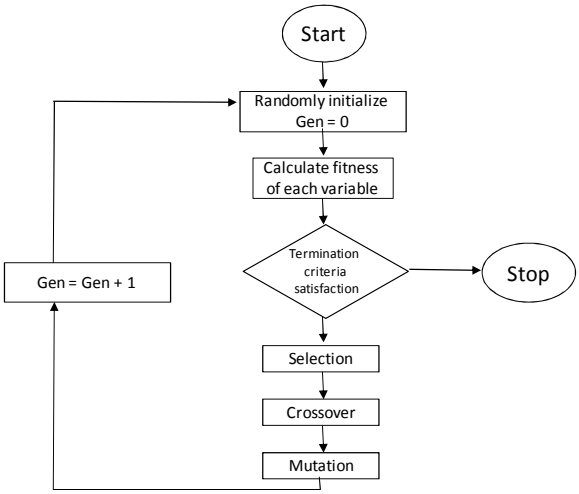

Figure 2 - Flow chart of simple genetic algorithms.

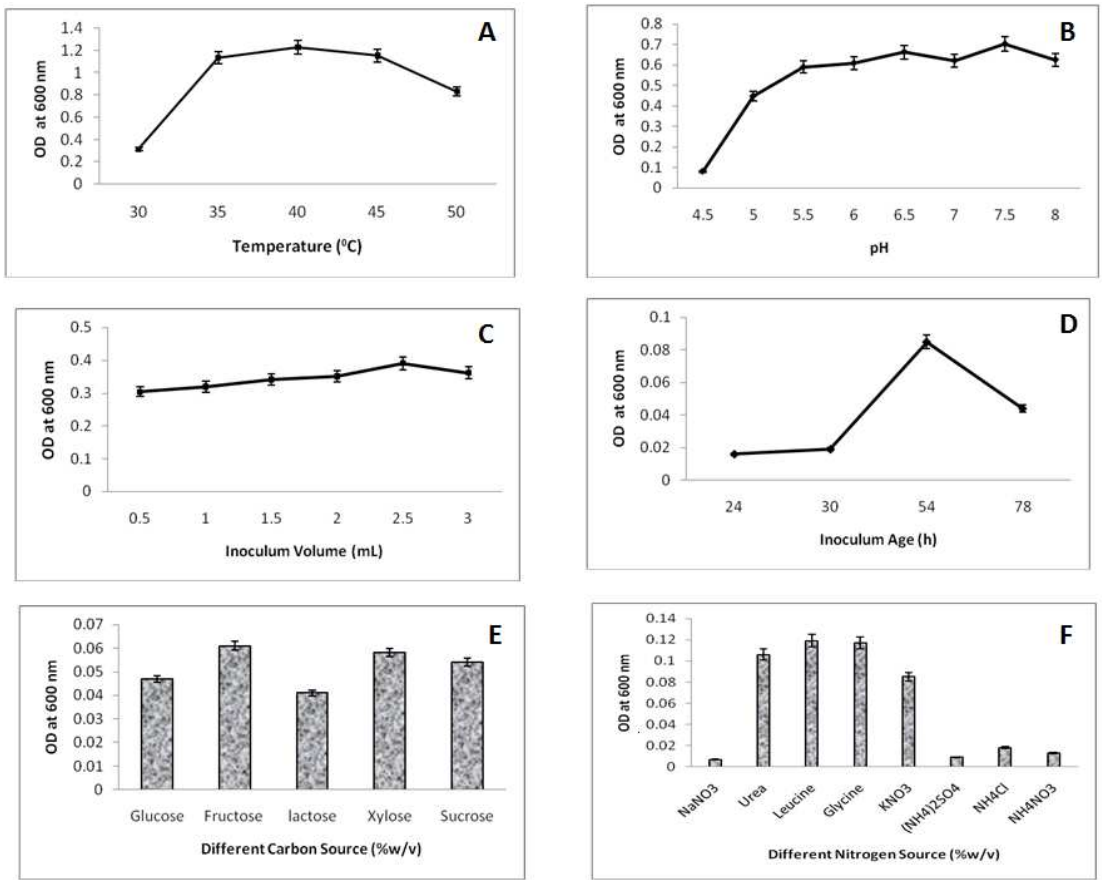

Figure 3 - Selection of different parameters for *B. bifidum* 255 growth (**A**. Selection of initial temperature for *B. bifidum* 255 growth, **B.** Selection of initial pH for *B. bifidum* 255 growth, **C.** Selection of initial inoculum volume for *B. bifidum* 255 growth, **D.** Selection of initial incubation period for *B. bifidum* 255 growth, **E.** Selection of suitable carbon source for *B. bifidum* 255 growth and **F.** Selection of suitable nitrogen source for growth).

Table 1 - Values of different parameters for single parameter optimization.

Different growth parameters	Variation of parameters	Maximum growth on parameter
Temperature, (°C)	30, 35, 37, 40, 45	37
pH	4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0 ,7.5, 8.0,	7.5
Inoculum volume, (ml)	0.5, 1.0, 1.5, 2.0, 2.5, 3.0	2.5
Inoculum age, (h)	24, 30, 54, 78	54
Carbon sources, (% w/v)	Glucose, Fructose, Sucrose Lactose, Xylose	Fructose
Nitrogen sources, (%w/v)	Sodium nitrate, Urea, Leucine, Glycine, Potassium nitrate, Ammonium sulphate, Ammonium chloride, Ammonium nitrate	Leucine

Empirical model development

From the above results, the maximum and minimum values of six independent parameters for *B. bifidum* were fixed as shown in Table 2. For developing the model between coded values x_1, x_2, x_3, x_4, x_5, x_6 of independent variables and dependent variable *Yp*, the experiments were conducted according to the fractional factorial design. All these combinations have been given in Table 3 with their corresponding *l* and *s* values. Various combination of process variable found at s_1=0, s_2=0 is shown in the Table 4 with their experimental value *Ye* for the growth of *B. bifidum.*

Table 2 - Limiting value of independent variables.

Parameters	Maximum value	Minimum value
Temperature, (°C)	40	30
pH	8	4.5
Inoculum volume, (ml)	3	0.5
Inoculum age, (h)	78	30
Carbon content, (%w/v)	42.06	30
Nitrogen content, (% w/v)	46.67	14

Table 3 - Values of *l* and *s* for various experimental runs with 6 independent variables as sacrificing interactions.

S. No.	x_1	x_2	x_3	X_4	x_5	x_6	l_1,s_1	l_2,s_2
1	1	1	1	1	1	1	5,1	5,1
2	1	1	1	1	1	-1	5,1	4,0
3	1	1	1	1	-1	1	4,0	4,0
4	1	1	1	1	-1	-1	4,0	3,1
5	1	1	1	-1	1	1	4,0	4,0
6	1	1	1	-1	1	-1	4,0	3,1
7	1	1	1	-1	-1	1	3,1	3,1
8	1	1	1	-1	-1	-1	3,1	2,0
9	1	1	-1	1	1	1	4,0	4,0
10	1	1	-1	1	1	-1	4,0	3,1
11	1	1	-1	1	-1	1	3,1	3,1
12	1	1	-1	1	-1	-1	3,1	2,0
13	1	1	-1	-1	1	1	3,1	3,1
14	1	1	-1	-1	1	-1	3,1	2,0
15	1	1	-1	-1	-1	1	2,0	2,0
16	1	1	-1	-1	-1	-1	2,0	1,1
17	1	-1	1	1	1	1	4,0	4,0
18	1	-1	1	1	1	-1	4,0	3,1
19	1	-1	1	1	-1	1	3,1	3,1
20	1	-1	1	1	-1	-1	3,1	2,0

Cont. table 3

(cont. table 3)

n	x1	x2	x3	X4	x5	x6	l1,s1	l2,s2
21	1	-1	1	-1	1	1	3,1	3,1
22	1	-1	1	-1	1	-1	3,1	2,0
23	1	-1	1	-1	-1	1	2,0	2,0
24	1	-1	1	-1	-1	-1	2,0	1,1
25	1	-1	-1	1	1	1	3,1	3,1
26	1	-1	-1	1	1	-1	3,1	2,0
27	1	-1	-1	1	-1	1	2,0	2,0
28	1	-1	-1	1	-1	-1	2,0	1,1
29	1	-1	-1	-1	1	1	2,0	2,0
30	1	-1	-1	-1	1	-1	2,0	1,1
31	1	-1	-1	-1	-1	1	1,1	1,1
32	1	-1	-1	-1	-1	-1	1,1	0,0
33	-1	1	1	1	1	1	4,0	5,1
34	-1	1	1	1	1	-1	4,0	4,0
35	-1	1	1	1	-1	1	3,1	4,0
36	-1	1	1	1	-1	-1	3,1	3,1
37	-1	1	1	-1	1	1	3,1	4,0
38	-1	1	1	-1	1	-1	3,1	3,1
39	-1	1	1	-1	-1	1	2,0	3,1
40	-1	1	1	-1	-1	-1	2,0	2,0
41	-1	1	-1	1	1	1	3,1	4,0
42	-1	1	-1	1	1	-1	3,1	3,1
43	-1	1	-1	1	-1	1	2,0	3,1
44	-1	1	-1	1	-1	-1	2,0	2,0
45	-1	1	-1	-1	1	1	2,0	3,1
46	-1	1	-1	-1	1	-1	2,0	2,0
47	-1	1	-1	-1	-1	1	1,1	2,0
48	-1	1	-1	-1	-1	-1	1,1	1,1
49	-1	-1	1	1	1	1	3,1	1,1
50	-1	-1	1	1	1	-1	3,1	3,1
51	-1	-1	1	1	-1	1	2,0	3,1
52	-1	-1	1	1	-1	-1	2,0	2,0
53	-1	-1	1	-1	1	1	2,0	3,1
54	-1	-1	1	-1	1	-1	2,0	2,0
55	-1	-1	1	-1	-1	1	1,1	2,0
56	-1	-1	1	-1	-1	-1	1,1	1,1
57	-1	-1	-1	1	1	1	2,0	3,1
58	-1	-1	-1	1	1	-1	2,0	2,0
59	-1	-1	-1	1	-1	1	1,1	2,0
60	-1	-1	-1	1	-1	-1	1,1	1,1
61	-1	-1	-1	-1	1	1	1,1	2,0
62	-1	-1	-1	-1	1	-1	1,1	1,1
63	-1	-1	-1	-1	-1	1	0,0	1,1
64	-1	-1	-1	-1	-1	-1	0,0	0,0

Table 4 - Experimental design for *B. bifidum* with experimental value *Ye*.

S. No.	Temp.°C (x_1)	pH (x_2)	Inoculum volume (ml) (x_3)	Inoculum age (h) (x_4)	Carbon content % (w/v) (x_5)	Nitrogen content % (w/v) (x_6)	Experimental value (*Ye*)
1	36.77	6.86	2.19	62.48	40.61	36.1	0.628
2	36.77	6.86	2.19	45.51	41.63	36.1	1.42
3	36.77	6.86	1.308	45.51	40.61	36.1	1.279
4	36.77	5.63	2.19	62.48	41.63	36.1	1.172
5	36.77	5.63	1.308	62.48	40.61	36.1	0.775
6	36.77	5.63	1.308	45.51	41.63	36.1	1.352
7	33.32	5.63	2.19	62.48	41.63	24.55	0.309
8	33.32	6.86	1.308	62.48	40.61	24.55	0.487
9	33.32	6.86	1.308	45.51	41.63	24.55	0.201
10	33.32	5.63	2.19	62.48	40.61	24.55	0.153
11	33.32	5.63	2.19	45.51	41.63	24.55	0.187
12	33.32	5.63	1.308	62.48	41.63	24.55	0.342
13	33.32	5.63	1.308	45.51	40.61	24.55	0.159
14	36.77	6.86	1.308	62.48	41.63	36.1	1.19
15	36.77	5.63	2.19	45.51	40.61	36.1	1.23
16	33.32	6.86	2.19	45.51	40.61	24.55	0.342
17	35	6.25	1.75	54	40.99	30.33	0.34
18	35	6.25	1.75	54	40.99	30.33	0.354
19	35	6.25	1.75	54	40.99	30.33	0.415
20	35	6.25	1.75	54	40.99	30.33	0.388
21	35	6.25	1.75	54	40.99	30.33	0.338
22	35	6.25	1.75	54	40.99	30.33	0.44
23	35	6.25	1.75	54	40.99	30.33	0.51
24	35	6.25	1.75	54	40.99	30.33	0.28
25	35	6.25	1.75	54	40.99	30.33	0.418
26	35	6.25	1.75	54	40.99	30.33	0.324
27	40	6.25	1.75	54	40.99	30.33	0.179
28	30	6.25	1.75	54	40.99	30.33	1.194
29	35	8	1.75	54	40.99	30.33	0.357
30	35	4.5	1.75	54	40.99	30.33	1.54
31	35	6.25	3	54	40.99	30.33	0.367
32	35	6.25	0.5	54	40.99	30.33	0.452
33	35	6.25	1.75	78	40.99	30.33	0.243
34	35	6.25	1.75	30	40.99	30.33	0.33
35	35	6.25	1.75	54	42.06	30.33	0.335
36	35	6.25	1.75	54	39.92	30.33	0.429
37	35	6.25	1.75	54	40.99	46.67	1.12
38	35	6.25	1.75	54	40.99	14	0.225

The experimental data were fitted to the full quadratic equation. The design matrix and the fitness of each term were analyzed by means of the ANOVA (Kumari et al., 2008). Figure 4 shows the corresponding model coefficients (R^2 0.840) together with the regression coefficient of determination, which is a measure of how well the regression model can be made to fit the raw data.

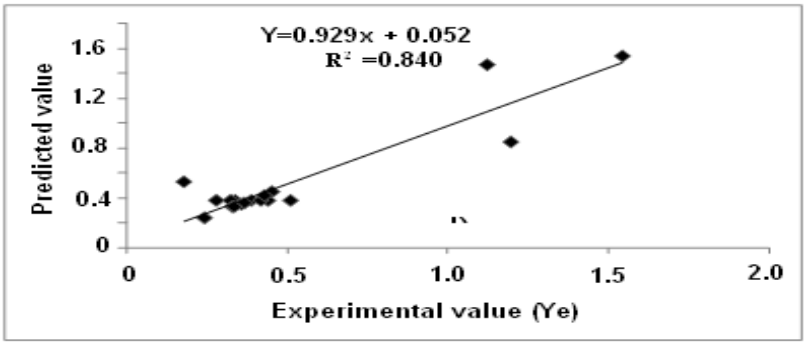

Figure 4 - Determination of regression equation Coefficient R^2 for *B. bifidum* 255 EMD method.

A self-organizing feature map network was used to predict the growth condition parameters. Different factors, *viz.* temperature, pH, inoculum volume, Inoculum age, additional carbon and nitrogen sources were used as each unit of input layer. The output layer was composed of one response variable, the growth of *B. bifidum*. A set of factors was used for training and fed into the computer. Several iterations were conducted with different numbers of neurons of hidden layer in order to determine the optimal ANN structure.

The optimum number of neurons in the hidden layer was iteratively determined by changing the number of neurons. This was started with two neurons and the number of neurons was increased up to six. The least MSE value and a good prediction of the outputs of both training and validation sets were obtained with four neurons in the hidden layer (Dutta et al., 2004). The R^2 value between the actual and estimated responses was determined as 0.930 (Fig. 5). In ANN modeling, the replicates at center point did not improve the rediction capability of the network because of the similar inputs.

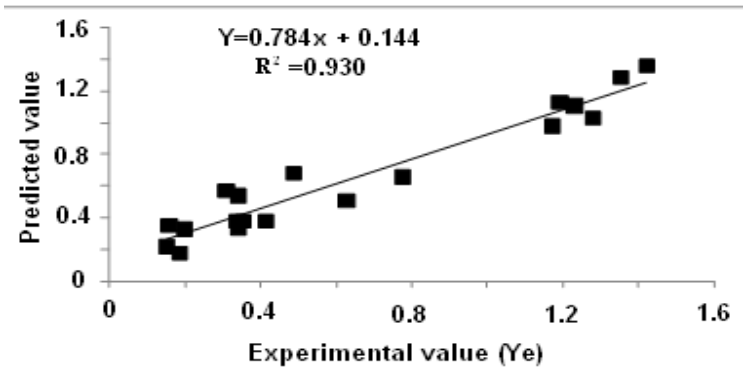

Figure 5 - Determination of regression equation Coefficient R^2 for *B. bifidum* 255 ANN method.

Using MATLAB 7.0, the constants of regression equation and predicted value of dependent variable (OD) were found out. The 'model' which was obtained for *B. bifidum* 255 is given below.

$$Yp = 0.3789 - 0.1621\ x_1 - 0.6200\ x_2 - 0.0451x_3 - 0.0414\ x_4 - 0.0567x_5 + 0.8012\ x_6 + 0.3110\ x_1^2 + 0.5667\ x_1x_2 + 0.0277x_1x_3 - 0.0953\ x_1x_4 - 0.0020\ x_1x_5 + 0.2900\ x_1x_6 + 94.3853\ x_2^2 + 8.4113\ x_2x_3 - 8.3785\ x_2x_4 + 32.7978\ x_2x_5 + 1.7702\ x_2x_6 + 0.3975\ x_3^2 + 0.6130\ x_3x_4 + 0.3763\ x_3x_5 - 3.0558\ x_3x_6 + 0.0620\ x_4^2 + 0.4437\ x_4x_5 - 8.6072\ x_4x_6 + 0.6171x_5^2 + 6.7946\ x_5x_6 - 31.6387\ x_6^2 \quad \text{(Eq. 2)}$$

The predicted value of independent variable and corresponding experimental value for *B. bifidum 255* is shown in Table 5. Genetic algorithms were applied on the data obtained from the neural network using MATLAB 7.0 The optimum values or the combination of different process parameters on which the bacterial growth measured by the optical density (OD) was maximum for *B. bifidum* which is given in the Table 6.

Table 5 - Experimental and predicted values for *B .bifidum* using RSM and ANN.

Using ANN		Using RSM	
Experimental values *Ye*	**Predicted values *Yp***	**Experimental values *Ye***	**Predicted values *Yp***
0.628	0.51	0.388	0.38
1.42	1.36	0.338	0.38
1.279	1.03	0.44	0.38
1.172	0.98	0.51	0.38
0.775	0.66	0.28	0.38
1.352	1.29	0.418	0.38
0.309	0.57	0.324	0.38
0.487	0.68	0.179	0.53
0.201	0.33	1.194	0.85
0.153	0.22	0.357	0.35
0.187	0.18	1.54	1.54
0.342	0.34	0.367	0.36
0.159	0.35	0.452	0.45
1.19	1.13	0.243	0.24
1.23	1.11	0.33	0.33
0.342	0.54	0.335	0.33
0.34	0.38	0.429	0.42
0.354	0.38	1.12	1.47
0.415	0.38	0.225	0.13

Table 6 - Optimum value of process parameters for *B. bifidum 255*.

Parameters	Optimum values
Temperature, (°C)	37.4
pH	7.09
Inoculums volume, (ml)	1.95
Inoculums age, (h)	58.18
Carbon content, (%) w/v	41.74
Nitrogen content, (%) w/v	46.23

There are several reports on the optimization of growth of the probiotic bacteria which are very close to the present result. Kiviharju et al. (2005) reported maximum production of *B.longum* at 40^{o}C. Ram and Chander, (2003) reported maximum growth of *Bifidobacteria* at 37 ^{o}C and pH 7.0. Laxmi et al. (2011) reported the addition of carbon and nitrogen sources for enhanced growth of *Bifidobacterium* sp. In the present study, the RSM/ANN coupled with GA methodology resulted in an enhanced biomass yield. This is a new approach not reported earlier. However, optimization studies based on the ANN-GA for improved performance of biological systems have been reported earlier by Haider et al. (2008) and Sivapathasekaran et al. (2010).

CONCLUSION

In the present study, MATLAB 7.0 was used to fit the experimental values into a regression equation which predicted the yield of *B. bifidum 255*. The RSM and ANN methodologies coupled with GA were used for optimizing the input parameters. Both the models provided similar quality predictions for the above independent variables in terms of the growth conditions with ANN with more accuracy in estimation. The regression coefficients (R^2) of ANN and RSM were 0.9368 and 0.8838, respectively, which clearly reflected that the ANN was better than RSM. The optimum values obtained after the GA study were 37.4°C, pH 7.09, inoculum volume 1.97 ml, inoculum age

58.58 h, carbon content 41.74% (w/v), nitrogen content 46.23% (w/v), resulting the maximum yield of probiotic bacteria. It was further noticed that ANN coupled with GA was the best combination for model development of *B.bifidum.*

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