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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 Mixed Culture of *Bifidobacterium bifidum* and *Lactobacillus acidophilus* using Response Surface Methodology and Artificial Neural Network

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ABSTRACT

Different culture conditions viz. additional carbon and nitrogen content, inoculum size and age, temperature and pH of the mixed culture of Bifidobacterium bifidum *and* Lactobacillus acidophilus *were optimized using response surface methodology (RSM) and artificial neural network (ANN). Kinetic growth models were fitted for the cultivations using a Fractional Factorial (FF) design experiments for different variables. This novel concept of combining the optimization and modeling presented different optimal conditions for the mixture of* B. bifidum *and* L. acidophilus *growth from their one variable at-a-time (OVAT) optimization study. Through these statistical tools, the product yield (cell mass) of the mixture of* B. bifidum *and* L. acidophilus *was increased. Regression coefficients (R^2) of both the statistical tools predicted that ANN was better than RSM and the regression equation was solved with the help of genetic algorithms (GA). The normalized percentage mean squared error obtained from the ANN and RSM models were 0.08 and 0.3%, respectively. The optimum conditions for the maximum biomass yield were at temperature 38°C, pH 6.5, inoculum volume 1.60 mL, inoculum age 30 h, carbon content 42.31% (w/v), and nitrogen content 14.20% (w/v). The results demonstrated a higher prediction accuracy of ANN compared to RSM.*

Key words: Response surface methodology (RSM), Artificial neural network (ANN), Genetic algorithms (GA), Fractional factorial design (FFD), *Bifidobacterium bifidum*, *Lactobacillus acidophilus*

INTRODUCTION

According to the recently adopted definition by FAO/WHO, probiotics are live microorganisms, which when administered in adequate amounts confer a health benefit on the host (Hemaiswarya et al. 2013). Probiotics are not a new invention but have existed in traditional foods such as beverages, salty fishes, yogurt, different types of cheeses, etc since olden times (Amara and shibl 2013). Several microbial groups have the potential to function as probiotics but the species of *Lactobacillus* and *Bifidobacterium* are the most commonly used as probiotics, including certain yeast and bacilli (Singh et al. 2011). Most bacterial species of this class are formally classified as GRAS (generally recognized as safe) organisms. The popularity of these microbes is based on the millennia of use in food and feed that are used in probiotic dairy drinks and yoghurts since hundreds of years (Sanders 1999). Currently, consumers are very much concerned about the sensorial, nutritional and functional attributes of food worldwide. A number of health benefits, which

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may be direct or indirect such as enhanced barrier function, modulation of the mucosal immune system, production of antimicrobial agents, enhancement of digestion and absorption of food and alteration of the intestinal microflora (Hemaiswarya et al. 2013), anti-mutagenic effects, anti-carcinogenic properties, improvement in lactose metabolism, reduction in serum cholesterol and immune system stimulation (Shah 2007), better calcium absorption, prevention of allergy and reduction in dental decay are associated with the consumption of probiotics (Singh et al. 2011).

According to a recent market research report, 'Probiotics Market (2009;2014)', the global probiotics market generated US$15.9 billion in 2008 and was expected to be worth US$ 32.6 billion by 2014 with a compound annual growth rate of 12.6% from 2009 to 2014. India is fast emerging as a potential market for probiotics in foods with several companies such as Nestle, Mother Dairy, Danisco, Chr Hansen, Yakult and Danone. The probiotic product industry in India is an estimated ₹ 20.6 million with a projected annual growth rate of 22.6% until 2015 (Ganguly et al. 2011). Probiotics in India generally come in two forms, viz., milk and fermented milk products with the former constituting a major chunk (50-60%) of the market. List of probiotic containing foods is wide and still growing. Main products existing in the market are dairy-based, including fermented milks, cheese, ice cream, buttermilk, milk powder, and yogurts, the latter accounting for the largest share of sales. Usually, dairy products are known as the 'best carrier' of probiotics.

Probiotic bacteria such as *Lactobacillus* and *Bifidobacterium* can play an important role in promoting human health in the gastrointestinal tract (Mitsuoka 1990). They actively contribute in the digestion, immune stimulation and inhibition of pathogens such as *Bacteroides, Escherichia, Clostriduim* and *Proteus,* which are potentially harmful bacteria found in the gastrointestinal tract (Ziemer and Gibson 1998). Recently, systematic reviews on the health benefits of probiotics in details have been published (Aureli et al. 2011; Amara and Shibl 2013). The effectiveness of the probiotic effects generally depends on the mechanisms by which they exerts their activities. Mostly, to treat a disease, the probiotics follow a set of mechanisms, which were recently reviewed (Hemaiswarya et al. 2013). The primary mechanism for probiotic action is known as competitive colonization, or competitive suppression, best described as the proliferation of probiotic bacteria in the human intestine, leaving little space and food for the growth of any pathogens. Secondary, the by-products (i.e., lactic acid and acetic acid) secreted by these probiotics lowers the pH, thereby creating a hostile environment for the growth of pathogenic microorganisms. The secreted acids also increases the peristalsis, which also helps to speed pathogens out from the intestines (Ballongue 1992; Biavati et al. 2000).

Traditional, i.e., one variable at-a-time (OVAT) method of bacterial growth optimization is not only time consuming but also neglects interactions of different variables, which affects the yield. Process optimization through 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 such as 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 to study the optimal process parameters that produce a maximum, or minimum value of the response and represents the direct and interactive effects of the process parameters through two and three-dimensional plots (Gangadharan et al. 2008). ANN is computational model of nervous systems. Natural organisms, however, do not possess only nervous systems but also have 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 a process, called 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 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. GA is a tool by which the optimization problems can be accurately solved with in a limited use of computer time (Das 2005). Optimization of various bacterial strains in Erlenmeyer flasks using different optimization

tools have been reported by several other authors (Nagarjun et al. 2005; Kumari et al. 2009; Lima et al. 2009; Negi and Banerjee, 2009; Usmiati and Marwati, 2009; Coelho et al. 2011; Meena et al. 2011; Meena et al. 2013).

This study developed the empirical model to increase the cell growth of the mixed culture of *B. bifidum* and *L. acidophilus* (1:1 ratio) by optimizing the growth parameters such as temperature, pH, inoculum volume, incubation period and additional effect of different carbon and nitrogen sources employing RSM, ANN and GA.

MATERIALS AND METHODS

Micro-organisms and their growth conditions

Bifidum bifidum NCDC 255 and *L. acidophilus* NCDC 14 were obtained in freeze dried state from National Collection of Dairy Cultures (NCDC), National Dairy Research institute, Karnal, Haryana, India. The methods used for the microbial culture activation and pellet extraction was same as earlier reported (Meena et al. 2011; Meena et al. 2013). Composition of the medium used for the growth of mixed culture of *B. bifidum* and *L. acidophilus* (1:1) contained (g/L) casein peptone 10, yeast extract 5, sodium acetate 5, Tween 80 1, $MgSO_4$ 0.2, $MnSO_4$ 0.05, K_2HPO_4 2 and its pH was maintained at 7.0. The growth of the mixed culture was carried out in 250 mL Erlenmeyer flasks each containing 50 mL growth medium and maintained at 37 ^{0}C. The cell biomass was determined by measuring the optical density (OD) of the medium after 24 h at 600 nm. Before measuring the OD, the liquid containing cells were centrifuged and washed with sterile distilled water for two times to remove the adhering medium constituents. All the solvents and reagents used in the present study were procured from Merck, Germany.

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 the growth of mixed culture.

Empirical model development using RSM

In order to find the effect of different growth parameters on the predicted value of bacterial growth Y_p 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. Using this model, the predicted value of response was find out within the domain of limiting values of independent variables. For the different growth parameters, it was desired to develop a polynomial model between the mixed culture growth and growth parameters to develop the following relationship between the coded values of independent variables, i.e., temperature (x_1), pH (x_2), inoculum volume (x_3), inoculum age (x_4), carbon sources (x_5) and nitrogen sources (x_6) and dependent variable (cell mass of mixed culture, 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

Fractional factorial design is a method by which the numbers of experiments are considerably reduced. This is used for the screening of independent variables, which have large effect on the dependent variables (Das 2005). Using two levels (+1 and -1) factorial design, two values of *l* and *s* were found out for all the experimental runs. Here, the values of *l* and *s* for two scarifying interactions were l_1, s_1, and l_2, s_2, respectively. With the help of factorial design, different 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). All the experiments were conducted according to s_1= 0 and s_2 =0 design (Meena et al. 2011), during present investigation.

Optimization

Artificial neural network modeling

In this present investigation, a feed forward back propagation neural network (Meena et al. 2011; Meena et al. 2013) was used to evaluate its capability in cell mass yield prediction of mixed culture. In this process, ANN computed the error

between the desired (predicted) response and the actual (experimental) response. The number of neurons in input layer, hidden layer and output layer of this neural network were kept as 6, 11 and 1, respectively. This ANN was first trained with reported data of *B. bifidum* (Meena et al. 2011). After training, it was able to predict the cell mass yield of the mixed culture accurately through error minimization that was compared with the predicted value of cell mass yield obtained from RSM (Meena et al. 2013).

Genetic Algorithms

In order to maximize the cell mass yield of the mixed culture, GA was applied to the developed ANN based model (Meena et al. 2011; Meena et al. 2013) by monitoring above mentioned six growth parameters. It was posed as the minimization of problem associated with the optimization studies. Genetic optimization was continued till the maximum cell mass yield obtained.

Software used

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

RESULTS AND DISCUSSION

Selection of initial parameters

Different growth variables for mixed culture growth were selected by OVAT method and results showed in Figure 1 (A - F). All these parameters, their variation and optimum values are given in Table 1.

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	7.0
Inoculum Volume, (mL)	0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5	1.5
Inoculum age, (h)	12, 24, 36, 48	24
Carbon sources, (% w/v)	Glucose, Fructose, Sucrose Lactose, Xylose	Lactose
Nitrogen sources, (%w/v)	Sodium nitrate, Urea, Leucine, Glycine, Potassium nitrate, Ammonium sulphate, Ammonium chloride, Ammonium nitrate	Potassium nitrate

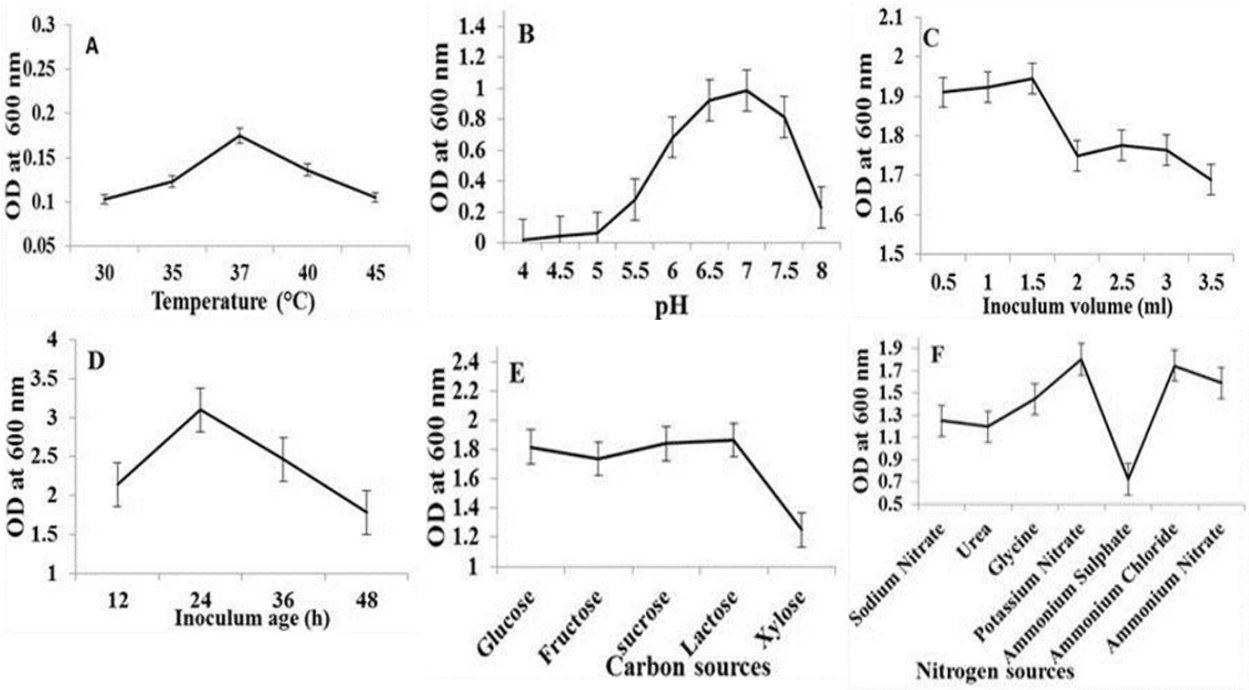

Figure 1 - Selection of different parameters for mixed culture growth (**A**. Selection of initial temperature, **B.** Selection of initial pH, **C.** Selection of initial inoculun volume, **D.** Selection of initial incubation period, **E.** Selection of suitable carbon source and **F.** Selection of suitable nitrogen source).

Empirical model development

From the initial parameter selection, the maximum and minimum values of six independent parameters for mixed culture were fixed as shown in Table 2. Then, a model was developed between the coded values of independent variables (x_1, x_2, x_3, x_4, x_5 and x_6) and dependent variable (Y_p) by conducting the experiments according to Fractional Factorial design. All these combinations are 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 growth of mixed culture.

Table 2 - Limiting value of independent variables.

Parameters	Maximum value	Minimum value
Temperature, (^{0}C)	45	30
pH	8.0	4.0
Inoculum volume, (mL)	3.5	0.5
Inoculum age, (h)	48	12
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 using $x_1x_2x_3x_4x_5$ and $x_2x_3x_4x_5x_6$ as sacrificing interactions.

Experiment No.	Coded values of independent variables						Sacrificing interactions	
	x_1	x_2	x_3	x_4	x_5	x_6	$x_1x_2x_3x_4x_5$ (l_1, s_1)	$x_2x_3x_4x_5x_6$ (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
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

(Cont. ...)

(Cont. Table 3)

Experiment No.	Coded values of independent variables						Sacrificing interactions	
	x_1	x_2	x_3	x_4	x_5	x_6	$x_1x_2x_3x_4x_5$ (l_1, s_1)	$x_2x_3x_4x_5x_6$ (l_2, s_2)
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 mixed culture of *B. bifidum* and *L. acidophilus* growth with experimental (Ye), RSM and ANN predicted values of mixed culture cell biomass.

Run order	Temp. ^{0}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 (Y_e), OD_{600}	RSM predicted values (Y_p)	ANN Predicted values Y_p
1	40.15	6.86	2.53	36.36	41.37	24.57	0.789	0.7412	0.828
2	40.15	6.86	2.53	23.64	40.61	24.57	0.2	0.3088	0.222
3	40.15	6.86	1.47	36.36	40.61	24.57	0.136	0.3512	0.1519
4	40.15	6.86	1.47	23.64	41.37	24.57	0.99	0.9081	1.022
5	40.15	5.63	2.53	36.36	40.61	24.57	0.345	0.4992	0.3777
6	40.15	5.63	2.53	23.64	41.37	24.57	0.845	0.7069	0.883
7	40.15	5.63	1.47	36.36	41.37	24.57	0.642	0.6086	0.6839
8	40.15	5.63	1.47	23.64	40.61	24.57	0.406	0.5267	0.443
9	34.85	6.86	2.53	36.36	40.61	36.11	0.746	0.85	0.787
10	34.85	6.86	2.53	23.64	41.37	36.11	0.985	0.7919	1.015
11	34.85	6.86	1.47	36.36	41.37	36.11	0.625	0.5383	0.666
12	34.85	6.86	1.47	23.64	40.61	36.11	0.825	0.8949	0.863
13	34.85	5.63	2.53	36.36	41.37	36.11	1.12	0.9772	1.142
14	34.85	5.63	2.53	23.64	40.61	36.11	0.926	0.9373	0.961
15	34.85	5.63	1.47	36.36	40.61	36.11	0.923	1.039	0.959
16	34.85	5.63	1.47	23.64	41.37	36.11	1.09	0.9137	1.114
17	37.5	6.25	2	30	40.99	30.54	0.708	0.75	0.72
18	37.5	6.25	2	30	40.99	30.54	0.619	0.67	0.563
19	37.5	6.25	2	30	40.99	30.54	0.758	0.8616	0.959
20	37.5	6.25	2	30	40.99	30.54	0.682	0.8616	0.959
21	37.5	6.25	2	30	40.99	30.54	0.912	0.8616	0.959
22	37.5	6.25	2	30	40.99	30.54	0.912	0.8616	0.959
23	37.5	6.25	2	30	40.99	30.54	0.98	0.8616	0.959
24	37.5	6.25	2	30	40.99	30.54	1.07	0.8616	0.959
25	37.5	6.25	2	30	40.99	30.54	0.995	0.8616	0.959
26	37.5	6.25	2	30	40.99	30.54	0.98	0.8616	0.959
27	45	6.25	2	30	40.99	30.34	0.953	0.8478	0.985
28	30	6.25	2	30	40.99	30.34	0.945	1.0502	0.979
29	37.5	8	2	30	40.99	30.34	0.001	0.067	0.003
30	37.5	4.5	2	30	40.99	30.34	0.27	0.3013	0.296
31	37.5	6.25	3.5	30	40.99	30.34	1	1.0507	1.03
32	37.5	6.25	0.5	30	40.99	30.34	1.09	1.0393	1.115
33	37.5	6.25	2	48	40.99	30.34	1.36	1.2615	1.335
34	37.5	6.25	2	12	40.99	30.34	1.3	1.3985	1.304
35	37.5	6.25	2	30	42.06	30.34	0.892	1.2116	0.93
36	37.5	6.25	2	30	39.92	30.34	1.11	0.9364	1.13
37	37.5	6.25	2	30	40.99	46.67	1.02	1.1252	1.05
38	37.5	6.25	2	30	40.99	14	0.624	0.5189	0.664

RSM is mainly used for the optimization of growth conditions, reaction parameter, or scaling-up the mixed culture growth conditions (Sen and Babu 2005). Experimental data were fitted to the full quadratic equation and the design matrix and the fitness of each term was analyzed by the means of ANOVA (Kumari et al. 2008). Figure 2 shows the corresponding model coefficients (R^2 0.7949) together with the regression coefficient of determination. This was a measure of how well the regression model could be made to fit the raw data. A self-organizing feature map network was used to predict the growth condition parameters using each independent variable as input layer and growth of mixed culture as response. The least Mean Squared Error (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 0.9591 (Fig. 3). In ANN modeling, the replicates at center point did not improve the prediction capability of the network because of the similar inputs.

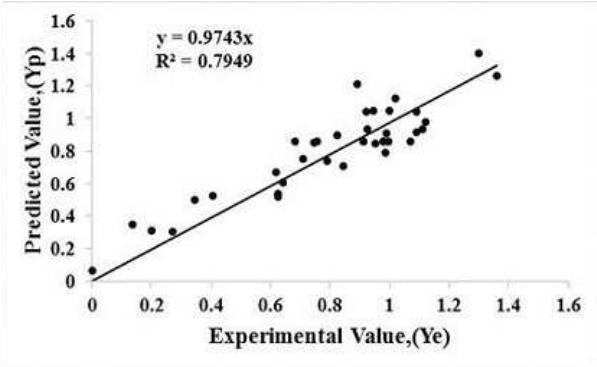

Figure 2 - Determination of regression equation coefficient R^2 for mixed culture cell biomass between experimental value (Ye) and RSM predicted values (Yp).

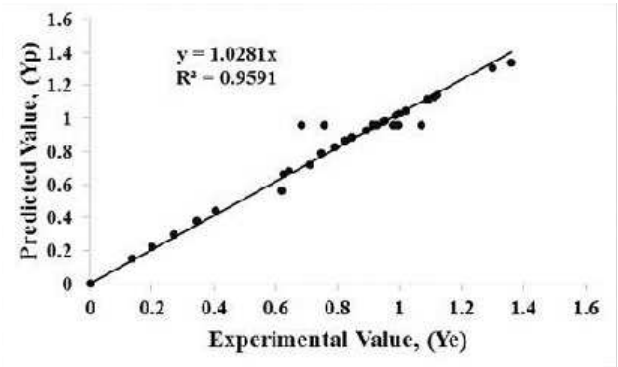

Figure 3 - Determination of regression equation coefficient R^2 for the mixed culture cell biomass between experimental value (Ye) and ANN predicted values (Yp).

Using MATLAB 7.0, the constants of regression equation and predicted value of dependent variable (OD) were found out. The obtained model for the mixed culture was as given below.

$$Y_p = 0.8578 - 0.1016x_1 - 0.1506\ x_2 + 0.0056x_3 - 0.0684x_4 + 0.1382x_5 + 0.3032x_6 + 0.0912x_1^2 - 0.7072x_2^2 + 0.1872x_3^2 + 0.4722x_4^2 + 0.2162x_5^2 - 0.0357x_6^2 + 0.3180x_1x_2 + 0.0676x_1x_3 - 0.2590\ x_1x_4 - 0.9292x_1x_5 + 1.3050x_1x_6 - 0.0168x_2x_3 - 0.2328x_2x_4 + 0.1855x_2x_5 - 0.0645x_2x_6 + 0.5151x_3x_4 + 0.2316x_3x_5 + 0.2224x_3x_6 - 0.2625x_4x_5 - .2625x_4x_6 - 1.8158x_5x_6$$ (Eq. 2)

The predicted value of independent variable and corresponding experimental value for the mixed culture is shown in the Table 5. Genetic algorithms were applied on the data obtained from neural network using MATLAB7.0, which yielded similar results as of ANN but in very short time.

Table 5 showed the optimum value, or combination of different process parameters on which the bacterial growth measured by optical density (OD) was highest for the mixed culture.

Table 5 - ANN and GA optimized values of the process parameters for maximum cell biomass of the mixed culture of *B. bifidum* and *L. acidophilus.*

Process parameters	Optimum values
Temperature, (°C)	38
pH	6.5
Inoculums volume, (mL)	1.60
Inoculum age, (h)	30
Carbon content, (%) w/v	42.31
Nitrogen content, (%) w/v	14.20

In the present study, RSM, ANN and GA optimization methodologies were used to predict the growth model of the mixed culture of *B. bifidum* and *L. acidophilus* (1:1) and optimized the growth parameters. Both the models were capable to predict the combination of independent variables for maximum cell biomass of mixed

culture but ANN showing more accuracy in estimation.

Regression coefficient (R^2) of ANN and RSM reflected that ANN was better than RSM. RSM was useful in getting insight information (e.g., interactions between different components) of the system directly, but ANN was also equally useful in the sensitivity analysis. ANN showed better modeling technique for data set showing nonlinear relationship over RSM. Thus, ANN could be a very powerful and flexible tool well suited for the development of empirical growth model due to an implicit corrective action arising from the training methodology and the associated estimation procedure. GA optimization results were similar to ANN but delivered within shortest use of computer time as compared to RSM and ANN. Present results showed that the higher cell mass yield of mixed culture was observed at 38°C, pH 6.5, inoculum volume and age 1.6 mL and 30 h, respectively, carbon content 42.31% (w/v) and nitrogen content (14.20%, w/v). This combination of independent variables could be of significant importance to starter culture producing industries in order to scale- up the production of *B. bifidum* and *L. acidophilus* on commercial scale more economically due to high cell mass yield.

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