Indian Journal of Chemical Technology
Vol. 23, September, 2016 pp. 335-344

# Multiobjective simultaneous optimization of biosurfactant process medium by integrating differential evolution with artificial neural networks

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*Received 27 May 2014; accepted 11 September 2015*

A method of differential evolution (DE) integrated with artificial neural networks (ANN) is derived for modelling and optimization of a biosurfactant process producing rhamnolipid by *Pseudomonas aeruginosa*. A central composite rotatable design (CCRD) data is used to develop multiple regression and ANN response surface models in order to integrate them with DE for optimizing the medium compositions. The DE with global search operators explores the search space of the response surface models and finds the optimum medium compositions that maximize the rhamnolipid productivity. A multiobjective simultaneous optimization strategy that integrates ANN model with DE search is found to compromise for biomass concentration and maximize the rhamnolipid activity as 55.9 mg/L ($R^2$ = 0.914) with an optimized medium compositions of glucose=24.079; $NH_4NO_3$=3.28; $KH_2PO_4$=0.24; yeast extract=7.95 and $MgSO_4.7H_2O$=2.69. The experimental rhamnolipid activity of 56 mg/L obtained using the optimized medium compositions are close to the predicted rhamnolipid activity. These findings demonstrate that the ANN-DE integrated multi objective optimization strategy is quite effective for simultaneous optimization of biochemical and biotechnological processes.

**Keywords:** Optimization, Rhamnolipid, *Pseudomonas* sp., Response surface methodology, Central composite design, Differential evolution

The amphiphilic compounds that exhibit high surface and emulsifying activity are known as biosurfactants. Biosurfactants uses various carbohydrate sources, oils and biomass wastes and are produced extra cellularly or as a part of the cell membrane by a variety of microorganisms. These products have gained a considerable interest in recent times mainly due to their high surface activities, heterogeneity and great potential for therapeutic applications like antimicrobial, antifungal, antioxidant and antiviral agents[1-8]. Among various biosurfactants, rhamnolipids are gaining much importance because of their applications in food, cosmetic and pharma industries. Response surface methodology (RSM) is the method that uses factorial designs and regression analysis along with optimization studies can serve to enhance the desired productivity of bio surfactants. RSM is a collection of statistical techniques for designing experiments, building models and evaluating the effects of factors. RSM involves establishing mathematical relations between the design variables and the resulting responses, and optimizing the process conditions. This methodology has been applied for response surface modeling and optimization of different biochemical and biosurfactant processes[9-11]. RSM is not free from problems when it is applied to multi-factor and multi-response situations. One of the difficulties associated with RSM is to understand the actual relationship between causal factors and individual responses. Another limitation is that a desirable condition for one response property is not always desirable for the other characteristics. This leads to the problem of conflicting objectives. Therefore, RSM need to be configured appropriately along with an efficient optimization methodology to deal with the problem of simultaneous optimization[12-20]. The aim of this work is to optimize the culture medium composition for rhamnolipid production by *Pseudomonas aeruginosa*. In this process, the factors include carbon source, nitrogen source, phosphate ratio and iron, and the responses are biomass and rahmnolipid concentrations. The objective is to maximize the Rhamnolipid concentration while compromising biomass concentration. Central composite rotatable design (CCRD) data is used to develop second order polynomial models with interaction terms. The model coefficients are tested for statistical significance,

insignificant coefficients are eliminated and the adequacies of the resulting models are validated for goodness of fit. Other mathematical approaches that combine evolutionary algorithms with artificial neural networks (ANN) are found superior to statistical approaches such as response surface methodology[12]. DE is an evolutionary optimization technique that works based on natural selection and survival of the fittest concepts of the biological world. Although differential evolution (DE) combined ANN has gained popularity for its use in various engineering fields, this modelling and optimization approach has not been fully exploited to enhance the production of bio molecules such as biosurfactants. The demand for rhamnolipid will increase several fold in coming years due to its potential application in the food processing industry in addition to clinical applications. Hence it is important to develop an economically viable bioprocess for production of rhamnolipid. In this study, an attempt was made to optimize the biosurfactant production by using DE integrated ANN based nonlinear modeling and optimization[12-14]. A CCRD based experimental data was used to develop ANN model. The ANN model was coupled with DE to find the maximum production level and the optimum concentrations of the critical medium components that affect significantly the production process. DE is first is first combined with ANN models to establish the biosurfactant process media compositions for maximizing the rhamnolipid concentration and minimizing the biomass concentration individually. These individual responses are further treated through a weighted objective function to optimize the media composition that satisfies the simultaneous optimization problem of the bio surfactant process. The results evaluated by RSM coupled with DE are compared with those of DE integrated ANN.

## Experimental Section

### Microorganism, medium, production and quantification

*Pseudomonas aeruginosa* obtained as a lyophilized culture from IMTECH was cultivated in a mineral base medium with the following composition (g/L): glucose=10.0; $NH_4NO_3$=1.7; $KH_2PO_4$=3.0; yeast extract=5.0; $MgSO_4.7H_2O$=0.2; $Na_2HPO_4$=7.0; and 1 mL hexadecane. The culture was incubated at 30°C, 200 rpm for 24 h. *P. aeruginosa* was grown in 250 mL flask with 100 mL of minimal media at the same conditions for 48 h with 10% inoculum. The rhamnolipid activity along with surface tension of 32 designed experiments was studied. Before rhamnolipids are produced on an industrial scale, the process parameters must be optimized. Different ways to enhance the yield include (a) strain improvement, (b) medium development, (c) process optimization and (d) the use of alternative, inexpensive substrates. The present study is focussed on medium optimization to enhance rhamnolipid activity. The conventional method for medium optimization involves changing one variable at a time, keeping the other factors fixed at a specific set of conditions. This method may lead to unreliable results and wrong conclusions. Moreover, carrying out experiments with every possible combination of the variables is impractical, because of the large number of experiments. The critical media components that influence the biosurfactant production were identified by single-factor-at-a-time experiments. The range and levels of the four independent variables along with the central values for these critical media components are listed in (Table 1). Further, a CCRD was applied with five factors and the experimental design data is shown in Table 2.

Table 1 — The central composite rotatable design for optimization of five nutrients (each at five levels) for the production bio surfactant rhamnolipid

| Factors | Levels | | | | |
|---|---|---|---|---|---|
| | Lowest | Low | Center | High | Highest |
| ($x_1$)Glucose | 7 | 12 | 17 | 22 | 27 |
| ($x_2$) $NH_4NO_3$ | 0 | 1 | 2 | 3 | 4 |
| ($x_3$)Yeast extract | 0 | 5 | 10 | 15 | 20 |
| ($x_4$)$MgSO_4$. $7H_2O$ | 0.1 | 0.2 | 0.3 | 0.4 | 0.5 |
| ($x_5$)$KH_2PO_4$(%) | 2 | 3 | 4 | 5 | 6 |

### Response surface modelling

The RSM technique can improve product yields and provide closer confirmation of the output responses toward the nominal and target requirements. In recent years, RSM has played an important role in biotechnology and other fields. The first step in RSM is to find a suitable approximation for the true functional relationship between the response (Y) and the set of independent variables.

If there is curvature in the system, then a polynomial of high degree must be used, such as the second-order model:

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \beta_{ij} x_i x_j + \varepsilon$$

Table 2 — Experimental design and results of the Central composite Rotatable design (CCRD)

| | Experimental values | | | | | Coded values | | | | | Responses | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $x_1$ | $x_2$ | $x_3$ | $x_4$ | $x_5$ | $X_1$ | $X_2$ | $X_3$ | $X_4$ | $X_5$ | Biomass | Rhamnolipid | Surface tension |
| 1 | 12 | 2 | 0.2 | 0.2 | 5 | -1 | -1 | -1 | -1 | 1 | 5.2 | 28 | 46 |
| 2 | 22 | 2 | 0.2 | 0.2 | 3 | 1 | -1 | -1 | -1 | -1 | 3.2 | 2.8 | 45.93 |
| 3 | 12 | 2 | 0.2 | 0.2 | 3 | -1 | 1 | -1 | -1 | -1 | 3 | 2.5 | 45 |
| 4 | 12 | 2 | 0.2 | 0.2 | 5 | 1 | 1 | -1 | -1 | 1 | 2 | 2.3 | 49 |
| 5 | 22 | 4 | 0.4 | 0.2 | 3 | -1 | -1 | 1 | -1 | -1 | 2.2 | 3 | 47 |
| 6 | 12 | 4 | 0.4 | 0.2 | 5 | 1 | -1 | 1 | -1 | 1 | 2.8 | 4.4 | 48 |
| 7 | 22 | 4 | 0.4 | 0.2 | 5 | -1 | 1 | 1 | -1 | 1 | 2.4 | 5.3 | 45 |
| 8 | 12 | 4 | 0.4 | 0.2 | 3 | 1 | 1 | 1 | -1 | -1 | 4.2 | 5.4 | 49 |
| 9 | 22 | 2 | 0.2 | 0.4 | 3 | -1 | -1 | -1 | 1 | -1 | 0.4 | 6.9 | 47.6 |
| 10 | 12 | 2 | 0.2 | 0.4 | 5 | 1 | -1 | -1 | 1 | 1 | 1.8 | 47 | 37.65 |
| 11 | 22 | 2 | 0.2 | 0.4 | 5 | -1 | 1 | -1 | 1 | 1 | 2.2 | 37 | 39 |
| 12 | 12 | 2 | 0.2 | 0.4 | 3 | 1 | 1 | -1 | 1 | -1 | 4 | 33 | 43.6 |
| 13 | 22 | 4 | 0.4 | 0.4 | 5 | -1 | -1 | 1 | 1 | 1 | 2.2 | 43 | 38.7 |
| 14 | 12 | 4 | 0.4 | 0.4 | 3 | 1 | -1 | 1 | 1 | -1 | 2.4 | 46 | 36.56 |
| 15 | 22 | 4 | 0.4 | 0.4 | 3 | -1 | 1 | 1 | 1 | -1 | 3.4 | 45 | 34.65 |
| 16 | 12 | 4 | 0.4 | 0.4 | 5 | 1 | 1 | 1 | 1 | 1 | 8.6 | 56 | 30.57 |
| 17 | 7 | 3 | 0.3 | 0.3 | 4 | -2 | 0 | 0 | 0 | 0 | 4 | 38 | 38.95 |
| 18 | 27 | 3 | 0.3 | 0.3 | 4 | 2 | 0 | 0 | 0 | 0 | 4.2 | 25 | 48 |
| 19 | 17 | 1 | 0.3 | 0.3 | 4 | 0 | -2 | 0 | 0 | 0 | 6.8 | 49 | 35.74 |
| 20 | 17 | 5 | 0.3 | 0.3 | 4 | 0 | 2 | 0 | 0 | 0 | 2 | 3.4 | 44.47 |
| 21 | 17 | 3 | 0.1 | 0.3 | 4 | 0 | 0 | -2 | 0 | 0 | 2.6 | 3.2 | 47.5 |
| 22 | 17 | 3 | 0.5 | 0.3 | 4 | 0 | 0 | 2 | 0 | 0 | 1.8 | 3.1 | 48 |
| 23 | 17 | 3 | 0.3 | 0.1 | 4 | 0 | 0 | 0 | -2 | 0 | 1 | 2.8 | 47.6 |
| 24 | 17 | 3 | 0.3 | 0.5 | 4 | 0 | 0 | 0 | 2 | 0 | 1.2 | 2.5 | 46 |
| 25 | 17 | 3 | 0.3 | 0.3 | 2 | 0 | 0 | 0 | 0 | -2 | 1.2 | 2.6 | 47.3 |
| 26 | 17 | 3 | 0.3 | 0.3 | 6 | 0 | 0 | 0 | 0 | 2 | 1.4 | 2.4 | 47.35 |
| 27 | 17 | 3 | 0.3 | 0.3 | 4 | 0 | 0 | 0 | 0 | 0 | 1.2 | 2.5 | 46.64 |
| 28 | 17 | 3 | 0.3 | 0.3 | 4 | 0 | 0 | 0 | 0 | 0 | 1.4 | 2.3 | 48 |
| 29 | 17 | 3 | 0.3 | 0.3 | 4 | 0 | 0 | 0 | 0 | 0 | 1.2 | 2.3 | 47.2 |
| 30 | 17 | 3 | 0.3 | 0.3 | 4 | 0 | 0 | 0 | 0 | 0 | 1.4 | 2.2 | 47 |
| 31 | 17 | 3 | 0.3 | 0.3 | 4 | 0 | 0 | 0 | 0 | 0 | 1.2 | 2.3 | 45 |
| 32 | 17 | 3 | 0.3 | 0.3 | 4 | 0 | 0 | 0 | 0 | 0 | 1.6 | 2.3 | 46 |

where $\beta_{ii}$ represents the quadratic effect of the $i^{th}$ factor and $\beta_{ij}$ represents the cross product effect, or interaction effect, between the $i^{th}$ and $j^{th}$ factors, where $x_1$, $x_2$, . . ., $x_k$ are the independent variables, $\beta_0$ the constant coefficient, $k$ the linear effect of the $k^{th}$ factor coefficients and $\varepsilon$ is the error observed in the response $Y$. The goal of RSM is to find an approximating function for predicting future response and to determine factor values that optimize the response function. An important assumption is that the independent variables are continuous and controllable by experiments with negligible errors. The task then is to find a suitable approximation for the true functional relationship between independent variables and the response surface.

**Artificial neural networks (ANN)**

Artificial neural networks (ANNs) are computer systems developed to mimic the operations of the human brain by mathematically modeling its neuro-physiological structure. They consist of a large number of computational units connected in a massively parallel structure. These computational units are called neurons which replace the nerve cells in the brain and the strengths of the inter connections are represented by weights, in which the learned information is stored[14,15]. The MFFN provides a mapping between an input ($x$) and an output ($y$) through a nonlinear function $f$, *i.e.*, $y = f(x)$. The three layered MFFN has input, hidden and output layers, each layer comprising of its own nodes. All the nodes in the input layer are connected using weighted links to the hidden layer nodes; similar links exist between the hidden and output layer nodes. Usually, the input and hidden layers also contain a bias node possessing constant output of 1. The nodes in the input layer do not perform any numerical processing, whereas all numerical processing is done by the hidden and output layer nodes, and they are termed as active nodes.

***Training algorithm***

The problem of neural network training is to obtain a set of weights such that the prediction error defined by the difference between the networks predicted outputs and the desired outputs is minimized. The iterative training makes the network to recognize patterns in the data and creates an internal model, which provides predictions for the new input condition. The input to the network consists of n-dimensional vector $x_p$ and a unit bias. Each input is multiplied by a weight $w_{ij}$ and the products are summed to obtain the activation state

$$S_{pj}: S_{pj} = \sum_{i=1}^{N} w_{ij} x_{pi} + w_{N+1,j} \quad \text{... (1)}$$

The output of the hidden layer neuron $O_{pj}$ for sigmoid function is calculated as

$$O_{pj} = f(S_{pj}) = \frac{1}{1+e^{-S_{pj}}} \quad \text{... (2)}$$

where $f$ represents the differentiable and non-decreasing function. The output layer of a single hidden layer network performs the same calculations as above, except that the input vector $x_p$ is replaced by the hidden layer output $O_p$ and the corresponding weights $w_{jk}$:

$$S_{pk} = \sum_{i=1}^{M} w_{jk} O_{pi} + w_{M+1,k} \quad \text{... (3)}$$

$$O_{pk} = y_{pk} = \frac{1}{1+e^{-S_{pk}}} \quad \text{... (4)}$$

Similar calculations can be extended to networks containing more than one hidden layer.

A simple way of measuring the progress of learning is by defining the sum of squared error, $E_p$ for $p$ learning patterns. The set of training examples consists of $p$ input-output vector pairs ($x_p$, $d_p$). Weights are initially randomized. Thereafter, weights are adjusted soas to minimize the objective function $E(w)$, defined as the mean squared error between the prediction outputs, $y_{pk}$ and the target outputs, $d_{pk}$ for all the input patterns:

$$E(w) = \sum_{p=1}^{p} E_p \quad \text{... (5)}$$

Where $E_p$ is the sum of squared error with each training example,

$$E_P = \sum_{K=1}^{M} (d_{pk} - y_{pk})^2 \quad \text{... (6)}$$

The task of $E_p$, minimization is accomplished by training the network using a gradient descent technique such as generalized delta rule[9,10]. According to this rule, the error function $\delta_{pk}$ between the hidden layer neurons to the output layer neuron k is computed. The weight changes from hidden to input layer can be calculated in the same way. After the weights are updated, a new training example is randomly selected, and the procedure is repeated until satisfactory reduction of the objective function is achieved.

***Processing of information***

Network training is an iterative procedure that begins with initializing the weight matrix randomly. Network learning process involves two types of passes: a forward pass and a reverse pass. In the forward pass, an input pattern from the example data set is applied to the input nodes, the weighted sum of the inputs to the active node is calculated which is then transformed into output using a nonlinear activation function such as sigmoid function. The outputs of the hidden nodes computed in this manner form the inputs to the output layer nodes whose outputs are evaluated in a similar fashion. In the reverse pass, the pattern specific squared error defined in Eqn. (5) Is computed and used for updating the network weights in accordance with the gradient strategy. The weight updating procedure when repeated for all the patterns in the training set completes one iteration. For a given ANN based modeling problem, the number of nodes in the network input layer and output layer is dictated by the input-output dimensionality of the pattern being modeled. However, the number of hidden nodes is an adjustable structural parameter. If the network architecture contains more hidden units than necessary, it leads to an oversized network. To avoid over fitting of the network, the network simulations are to be conducted by systematically varying the number of hidden units. These simulations provide optimal network architecture with the smallest error magnitude for the test data. The neural network architecture for 5 input 2 output variables is given in the Fig. 1.

***Differential evolution (DE)***

Evolutionary algorithms are widely used to solve optimization problems in various fields. These algorithms have an advantage over conventional

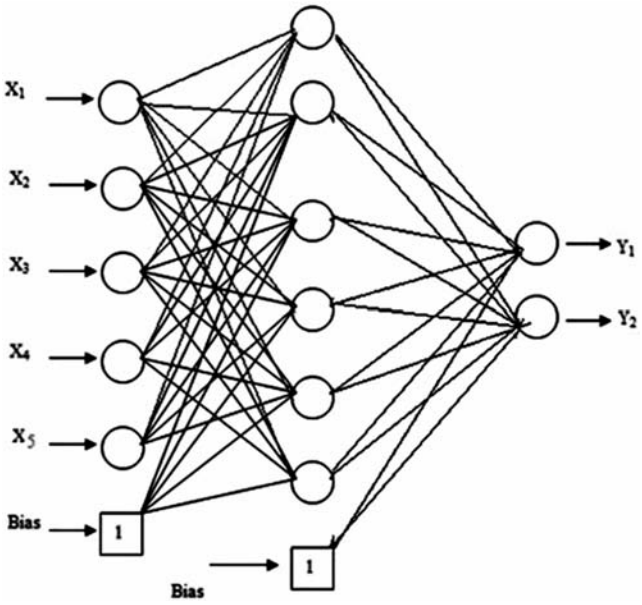


Fig. 1 — Artificial neural network architecture: Multi input multi output (MIMO) of 5 inputs with 2 outputs such as biomass and rhamnolipid activity.

gradient-based search procedures because they are capable offinding global optima of multi-modal functions and searching design spaces with disjoint feasible regions. Among the evolutionary algorithms, differential evolution (DE) is a simple population based search algorithm for global optimization of real valued functions. Its robustness and effectiveness has been demonstrated in a variety of applications. DE is controlled by three parameters, namely, population size(NP), the crossover operator (CR) and mutation constant called scaling factor (F). The performance of DE depends on the manipulation of target vector and difference vector in order to obtain a trial vector. The DE algorithm can be studied from[21,22] Store and Price *et al*., 1997. The first initial population of size PN vectors $[x_1^0, x_2^0,\ldots, x_{PN}^0]$ are generated at random in *D*-dimensional search space and the fitness function values are evaluated. DE extracts distance and direction information from the current vectors and adds random deviation for diversity to generate new parameter vectors. With the target vector and mutant vector, mutation has been performed. *F* is the mutation constant which controls the amplification of the difference between two individuals. After mutation, crossover is introduced to increase the diversity of the mutant vectors. During this operation, the trial vector is developed from the elements of target vector and the elements of the mutant vector is derived. *Cr* is the crossover constant in the range [0,1]. Finally, selection is performed by comparing the trail vector produced by the crossover operator with the target vector and the one with better fitness function is allowed to enter the next generation.

**Integration of DE with ANN**

It has been known that machine learning techniques such as ANN mimic different aspects of biological information processing for data modeling and could prove to be useful in media optimization for fermentation. Back propagation algorithm, a multilayer feed forward ANN, trains and then evaluates system performance using the adaptive gradient learning rule. The learning rate of the network was set to a value that resulted in an optimal coefficient of correlation ($R^2$) for the NN. Regression-based RSM requires the order of the model to be stated, while the ANN tends to implicitly match the input vector to the output vector. DE on the other hand, is a commonly used global enhancement technique which optimizes a given function over a particular range, and is based on the evolutionary methods of natural selection of the best individuals in a population. The DE explores all regions of the solution space using a population of individuals. Initially, all the population of individuals is generated randomly and mutation, crossover and selection processes are calculated. Then the fitness function evaluated an objective function. In the present work, the selected vectors are used for fitness function calculation using artificial neural networks. The flow chart of DE linked ANN is given in Fig 2.

**Simultaneous optimization strategy**

The maximization of a particular response can be achieved by input conditions that possibly will not satisfy the minimization requisite of other response. In ordinance to propitiate such incongruous goals over the identical stable optimal input media conditions, with a lonely function that ruminates concurring optimization of numerous response variables is obliged. Hence a customary of optimization created on the distance between the predicted value of each response and the optimum value of each response can be performed. Such a criterion is given by

$$D = \sqrt{\sum_{i=1}\left\{\left(\frac{FD_i - FO_i}{\sigma_i}\right)\right\}^2} \quad \ldots (7)$$

where D is the distance function, $\sigma_i$ is the standard deviation of the observed values for each response

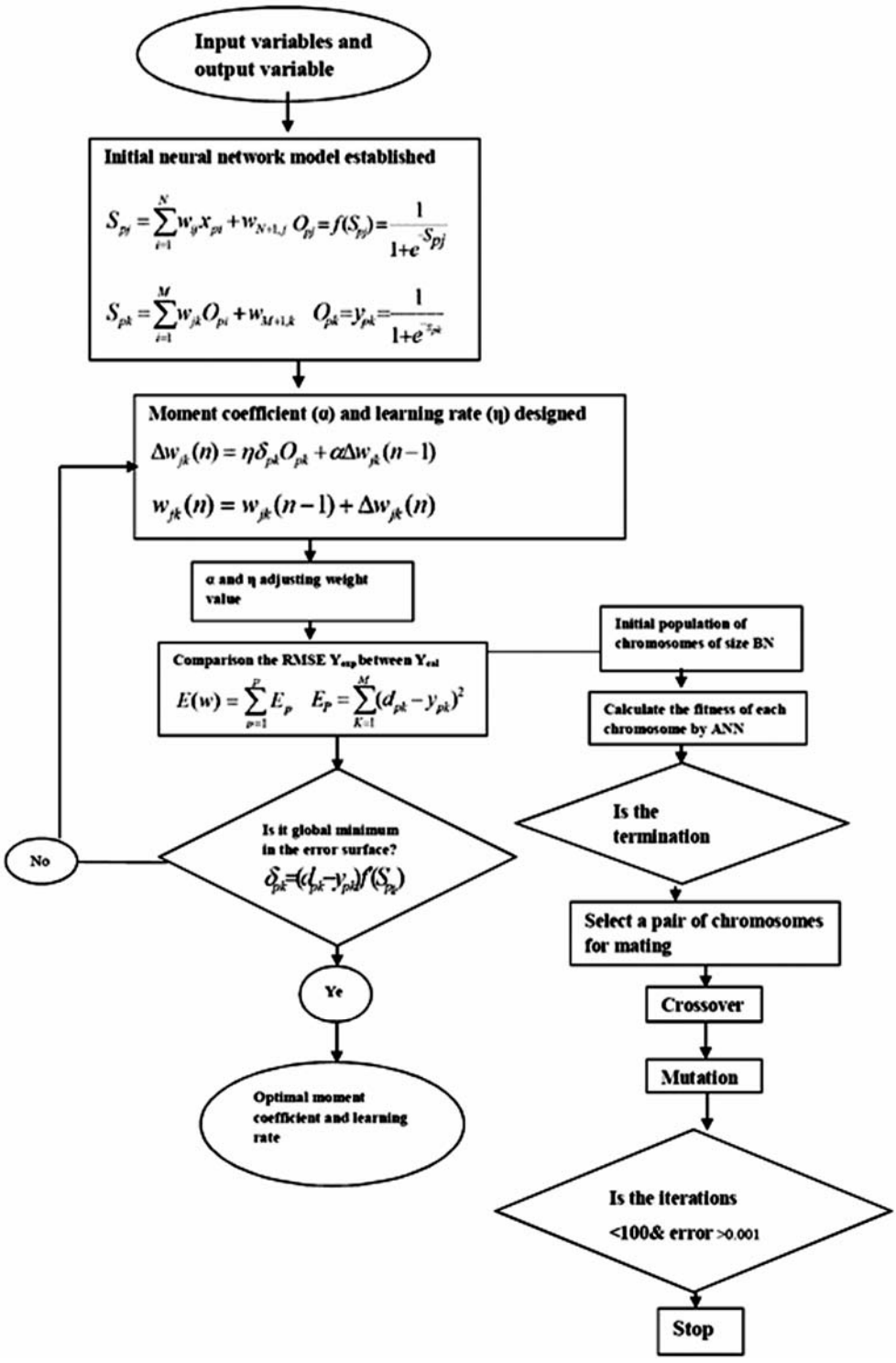


Fig. 2 — Flow chart of ANN-DE algorithm

variable, $FD_i$ is the ideal value of each response variable optimized individually over the experimental region and $FO_i$ is the predicted value of each response for the same set of casual factors.

## Results and Discussion

Experimentally analysed data indicates how the activity of biosurfactant varies with surface tension. A good bio surfactant reduces surface tension from

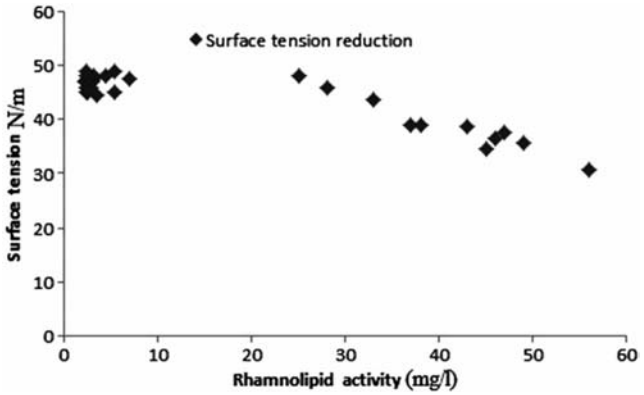


Fig. 3 — Surface tension reduction along with rhamnolipid activity observed for 48 h

70 to 30 dyn/cm. The better activity of biosurfactant can be observed from the experimental data of surface tension and rhamnolipid activity as plotted in Fig.3.

**Response surface methodology**

In this work, CCRD design data is used for fitting second-order models. According to this design, the total number of experiments required is $2^{k-1} + 2k + n_0$, which includes $2k$ factorial points with its origin at the center, $2k$ points fixed axially at a distance $\beta$ ($\beta = 2k/4$) from the center to generate the quadratic terms, and replicate tests at the center $(n0)$; where $k$ is the number of independent variables. A design should include enough replications, often at the centre points, to provide an independent estimate of the experimental error and allow for a test for lack of fit of the model. For five variables, the recommended number of tests at the centre is six. Hence the total number of tests required for five independent variables is $2^4 + (2\times5) + 6=32$.

For statistical calculation, the experimental variables $x_i$ has been coded as Xi according to the following transformation equation:

$$X_i = \frac{x_i - x_O}{\Delta x_i}$$

where $X_i$ is the dimensionless coded value of the $i^{th}$ independent variable, $x_i$ is the uncoded value of the $i^{th}$ independent variable, $x_0$ is the value of $x_i$ at the centre point, and $\Delta x_i$ is the step change value of the real variable $x_i$. The dependent variables are the biomass concentration ($y_1$, g/L) and rhamnolipid concentration ($y_2$, mg/L). The experimental design matrix with coded factors is shown in Table 2.

$$Y_{bio} = 1.259574 + 0.444681X_1 + 0 + 0.2X_3 + 0.016667X_4 + 0.2X_5 + 0.475X_{12} + 0.475X_{13} + 0.525X_{23} + 0.575X_{14} + 0.825X_{24} + 0.625X_{34} - 0.1X_{15} - 0.2X_{25} + 0.2X_{35} + 0.3X_{45} + 0.543085\,X_1^2 + 0.840426\,X_2^2 + 0.290426\,X_3^2 + 0.015426\,X_4^2 + 0.065426\,X_5^2 \quad \ldots (8)$$

$$Y_{rham} = 0.976596 + 0.632447X_1 - 3.575X_2 + 2.016667X_3 + 10.81667X_4 + 3.25X_5 - 0.775X_{12} + 0.3X_{13} + 1.575X_{23} + 4.625X_{14} + 3.175X_{24} + 5.225X_{34} - 2.0875X_{15} - 3.0625X_{25} - 3.7375X_{35} + 1.6125X_{45} + 8.319681\,X_1^2 + 7.310904\,X_1^2 + 1.548404\,X_1^2 + 1.423404\,X_1^2 + 1.385904\,X_5^2 \quad \ldots (9)$$

The comparison of the experimental responses with the model predictions plotted in Fig. 4 shows the prediction ability of the fitted regression models for both the biomass and rahmnolipid concentrations. The data in Table 1 is used to fit the regression models as Eqns 8 and 9. The statistical significance of the regression model coefficients is tested for 98% confidence level using student t-test. The t-values along with standard error for both biomass and rhamnolipid activity are given in Tables 3a and 3b. From the Tables 3a, 3b the insignificant coefficients are eliminated based on the variable selection. The P values are used to check the significance of each of the coefficients, which is necessary to understand the pattern of the mutual interactions between the independent variables. The lower the magnitude of P, the more significant is the corresponding coefficient. The significance of each coefficient was determined by student *t*-test and *P*-value, which is shown in Table 3 of column 4. The larger the magnitude of t-value and smaller the P-value, the more significant is the corresponding coefficient. After eliminating the insignificant coefficients, the regression equations for biomass and rhamnolipid concentrations in coded factors are represented by

$$Y_{bio} = 1.259574 + 0.444681X_1 + 0.016667X_4 + 0.2X_5 + 0.475X_{12} + 0.475X_{13} + 0.525X_{23} + 0.575X_{14} + 0.825X_{24} + 0.625X_{34} + 0.2X_{35} + 0.3X_{45} + 0.543085\,X_1^2 + 0.840426\,X_2^2 + 0.290426\,X_3^2 + 0.065426\,X_5^2 \quad \ldots (10)$$

$$Y_{rham} = 0.976596 + 0.632447X_1 - 0.775X_{12} + 0.3X_{13} + 1.575X_{23} + 4.625X_{14} + 3.175X_{24} + 5.225X_{34} + 1.6125X_{45} + 8.319681\,X_1^2 + 7.310904\,X_2^2 + 1.548404\,X_3^2 \quad \ldots (11)$$

Figure 4 shows the contour response surface plot of the model equation describing rhamnolipid activity.

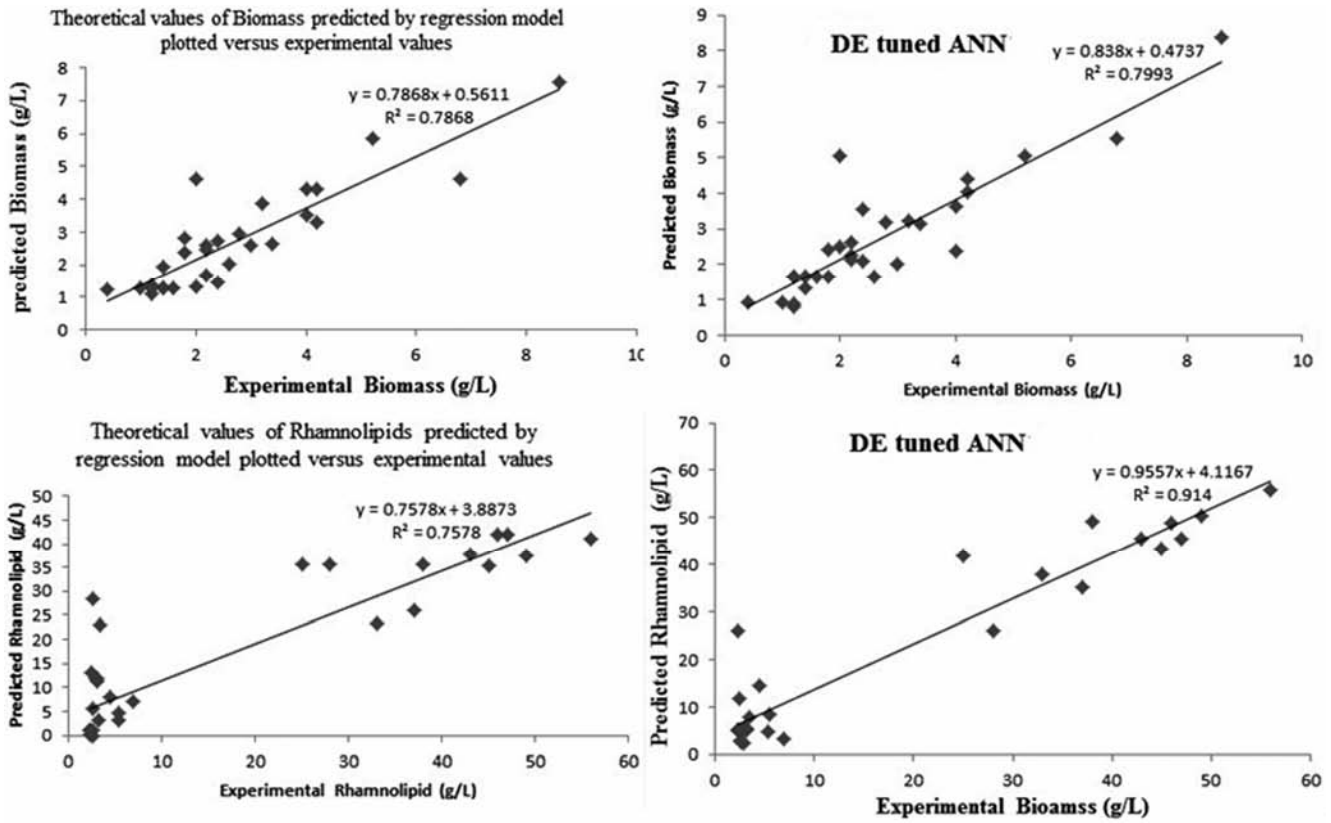


Fig. 4 — Experimental and model predicted values of response surface models and DE tuned ANN models of biomass and rhamnolipid activity.

Table 3a — Coefficient estimates in the regression model for biomass selected through variable selection biomass MSE=0.03125*0.00001

| Intercept | Coefficient estimate | Standard error | $t_{table} < t_{calculated}$; $t_{0.05,31} = 1.695$ | P-value |
|---|---|---|---|---|
| $X_0$ | 1.259574 | 0.078075 | 16.132827 | <0.01 |
| $X_1$ | 0.444681 | 0.047277 | 9.405878 | <0.005 |
| $X_2$ | 0 | 0.039896 | 0.000000 | <0.25 |
| $X_3$ | 0.2 | 0.039896 | 5.013072 | 0.0005 |
| $X_4$ | 0.016667 | 0.039896 | 0.417756 | <0.25 |
| $X_5$ | 0.2 | 0.039896 | 5.013072 | 0.0005 |
| $X_1X_2$ | 0.475 | 0.048862 | 9.721246 | <0.005 |
| $X_1X_3$ | 0.475 | 0.048862 | 9.721246 | <0.005 |
| $X_2X_3$ | 0.525 | 0.048862 | 10.744535 | <0.0005 |
| $X_1X_4$ | 0.575 | 0.048862 | 11.767824 | <0.0005 |
| $X_2X_4$ | 0.825 | 0.048862 | 16.884269 | <0.01 |
| $X_3X_4$ | 0.625 | 0.048862 | 12.791113 | 0.025 |
| $X_1X_5$ | -0.1 | 0.048862 | -2.046578 | 0.05 |
| $X_2X_5$ | -0.2 | 0.048862 | -4.093156 | 0.005 |
| $X_3X_5$ | 0.2 | 0.048862 | 4.093156 | 0.005 |
| $X_4X_5$ | 0.3 | 0.048862 | 6.139734 | 0.05 |
| $X_1^2$ | 0.543085 | 0.040943 | 13.264417 | 0.0025 |
| $X_2^2$ | 0.840426 | 0.036342 | 23.125429 | 0.001 |
| $X_3^2$ | 0.290426 | 0.036342 | 7.991446 | <0.001 |
| $X_4^2$ | 0.015426 | 0.036342 | 0.424454 | <0.25 |
| $X_5^2$ | 0.065426 | 0.036342 | 1.800271 | 0.1 |

Table 3b — Coefficient estimates in the regression model for rhamnolipid selected through variable selection MSE=0.00001

| Intercept | Coefficient estimate | Standard error | $t_{table} < t_{calculated}$; $t_{0.05,31} = 1.695$ | P-value |
|---|---|---|---|---|
| $X_0$ | 0.976596 | 0.246896 | 5.101648 | 0.0005 |
| $X_1$ | 0.632447 | 0.149503 | 2.974400 | 0.02 |
| $X_2$ | -3.575 | 0.126161 | 0.000000 | 0.0 |
| $X_3$ | 2.016667 | 0.126161 | 1.585273 | 0.1 |
| $X_4$ | 10.81667 | 0.126161 | 0.132106 | <0.25 |
| $X_5$ | 3.25 | 0.126161 | 1.585273 | 0.1 |
| $X_1X_2$ | -0.775 | 0.154515 | 3.074128 | 0.0025 |
| $X_1X_3$ | 0.3 | 0.154515 | 3.074128 | 0.0025 |
| $X_2X_3$ | 1.575 | 0.154515 | 3.397720 | 0.001 |
| $X_1X_4$ | 4.625 | 0.154515 | 3.721313 | 0.0005 |
| $X_2X_4$ | 3.175 | 0.154515 | 5.339275 | 0.0005 |
| $X_3X_4$ | 5.225 | 0.154515 | 4.044905 | 0.005 |
| $X_1X_5$ | -2.0875 | 0.154515 | -0.647185 | 0.25 |
| $X_2X_5$ | -3.0625 | 0.154515 | -1.294370 | 0.1 |
| $X_3X_5$ | -3.7375 | 0.154515 | 1.294370 | 0.1 |
| $X_4X_5$ | 1.6125 | 0.154515 | 1.941554 | 0.05 |
| $X_1$ | 8.319681 | 0.129473 | 4.194577 | 0.001 |
| $X_2$ | 7.310904 | 0.114924 | 7.312903 | <0.0005 |
| $X_3$ | 1.548404 | 0.114924 | 2.527117 | 0.025 |
| $X_4$ | 1.423404 | 0.114924 | 0.134224 | <0.25 |
| $X_5$ | 1.385904 | 0.114924 | 0.569296 | <0.25 |

The responses of rhamnolipid and biomass concentrations obtained from these prediction equations are integrated with DE to optimize the biosurfactant production medium compositions.

**DE integrated ANN**

Back propagation algorithm (BPA), a multilayer feed-forward ANN, was used to model the non-linear relationships between media components (Glucose, $NH_4NO_3$, yeast extract, $MgSO_4.7H_2O$ and $KH_2PO_4$) and rhamnolipid activity. Four neurons were used in the input layer, 12 in the hidden layer, and 2 in the output layer with sigmoid function as the transfer function to model the dependence of rhamnolipid production on media components. The five input variables (Glucose, $NH_4NO_3$; $KH_2PO_4$; $MgSO_4.7H_2O$; and yeast extract) and the 2 output variable (biomass and rhamnolipid activity) listed in Table 2 were used. Once the ANN model was developed, DE was applied to determine the values of biomass and rhamnolipid production and optimum concentration of the media components. DE was used with a population size of 30, mutation rate of 0.04, and uniform crossover rate of 0.7. The ANN prediction responses of rhamnolipid and biomass concentrations were integrated with DE to optimize biosurfactant production medium compositions that maximize the rhamnolipid activity while compromising biomass concentration.

**Multiobjective simultaneous optimization**

Simultaneous optimization was performed to evaluate the formulation conditions that optimize the individual objectives. Maximization of Rhamnolipid activity minimization of biomass concentration is considered as the desired objectives. The individually optimized responses were used in the performance criterion defined by Eqn. (7) to obtain a single set of conditions that simultaneously satisfy both the responses. The simultaneous optimization based on the minimization of distance function in Eqn. (7) has established optimum medium compositions that maximize the rhamnolipid activity while compromising for the biomass concentration.

The optimum concentrations of the media components were Glucose: 24.07, $NH_4NO_3$: 3.28, $KH_2PO_4$: 7.95, $MgSO_4.7H_2O$: 0.24 and yeast extract: 2.6915. These optimized medium composition yields the maximum rhamnolipid concentration as 56 mg/L along with the biomass concentration of 8.2 mg/L. The ANN-DE Multiobjective simultaneous optimization strategy provides better performance for predicting rhamnolipid activity ($R^2$=0.914) and biomass concentration ($R^2$=0.799) than the RSM-DE which provides the predictions of rhamnolipid activity ($R^2$= 0.7578) and biomass concentration ($R^2$=0.7868). The comparison of the experimental responses with the model predictions were plotted in Fig. 4. The predicted results obtained with the DE integrated RSM and ANN models were further validated experimentally. The results were found to be closer when compared with experimental results.

## Conclusion

The significant media components were optimized using an RSM and ANN integrated DE. The optimized medium compositions (g/L) of Glucose=24.079; $NH_4NO_3$=3.28; $KH_2PO_4$=0.24; yeast extract=7.95, $MgSO_4.7H_2O$ =2.69 were identified to be significant media components for in maximizing the rhamnolipid concentration to an extent of 56 mg/L by *Pseudomonas aerugenosa* in batch fermentation using a Central composite rotatable design (CCRD). The results of DE integrated ANN were experimentally validated. The optimized medium compositions by ANN-DE were found to be to be more effective in enhancing the rhamnolipid production. The results thus demonstrate the usefulness of DE integrated ANN strategy for optimization of rhamnolipid biosurfactant process.

## Acknowledgements

Financial assistance from DST through the grant SR/wos-a/ET-20/2009 is gratefully acknowledged.

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