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ORIGINAL ARTICLE

Modeling and optimization of tannase production with *Triphala* in packed bed reactor by response surface methodology, genetic algorithm, and artificial neural network

Subbalaxmi Selvaraj[1] · Ramachandra Murty Vytla[1] · G. S. Vijay[2] · Kannan Natarajan[1]

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Abstract
In this research, optimization of the production medium to enhance tannase production by *Bacillus gottheilii* M2S2 in laboratory-scale packed bed reactor was studied. Amount of substrate *Triphala*, moisture content, aeration rate, and fermentation period was chosen for optimization study. During one variable at a time optimization, the highest tannase activity of 0.226 U/gds was shown with *Triphala* as a substrate at the fermentation period of 32 h. Furthermore, the optimum conditions predicted by response surface methodology (RSM) and genetic algorithm (GA) were found to be 11.532 g of substrate *Triphala*, 47.071% of the moisture content, and 1.188 L/min of an aeration rate with uppermost tannase activity of 0.262 U/gds. In addition, the single hidden layer feedforward neural network (SLFNN) and the radial basis function neural network (RBFNN) of an artificial neural network (ANN) were adopted to compare the prediction performances of the RSM and GA. It revealed that the ANN models (SLFNN, $R^2 = 0.9930$; and RBFNN, $R^2 = 0.9949$) were better predictors than the RSM ($R^2 = 0.9864$). Finally, the validation experiment exhibited 0.265 U/gds of tannase activity at the optimized conditions, which is an 11-fold increase compared to unoptimized media in shake flask.

Keywords Tannase · Packed bed reactor · Genetic algorithm · Artificial neural network · Response surface methodology · *Triphala*

Introduction

Agricultural residues have been found to be feasible substrates for the production of value-added industrial enzymes (Pandey et al. 2001). *Triphala* is an agricultural residue which generally consists of equal proportions of three myrobalans, i.e., *Emblica officinalis*, *Terminalia bellirica,* and *Terminalia chebula*. The composition of tannic acid in these different solid substrates reported in the literature was (% w/w): *E. officinalis,* 28; *T. bellirica,* 17; *and T. chebula*, 30 (Bali et al. 2013). This mixed substrate *Triphala* has been used in our previous study for the production of a tannase (Subbalaxmi and Vytla 2017a, b), whereas individual substrates *T. chebula* and *E. officinalis* have been used independently for the synthesis of tannase (Prasanna et al. 2012; Selwal et al. 2010).

Tannin acyl hydrolase (E.C. 3.1.1.20) is commonly known as tannase, which is one of the most important industrial enzymes which acts on tannic acid and hydrolyzes to glucose and gallic acid. Tannase have found various applications in different industrial sectors such as food (fruit and vegetable juice clarification, deprivation of plant phenolics, and preparation of coffee flavor cold drinks and instant tea), chemical (treatment of effluents of textile and tannery), pharmaceutical (the products of tannase gallic acid and propyl gallate used in the synthesis of an antibacterial drug called trimethoprim and antioxidants, respectively), and beverage (removal of chill haze formation in the preparation of beer and wine) industries (Aithal and Belur 2013; Aguilar et al. 2007; Rout and Banerjee 2006).

A variety of bacteria are capable of producing enzyme tannase. *Bacillus* species are well known for their efficient production of a varied range of industrial microbial enzymes

✉ Ramachandra Murty Vytla
murty.vytla@manipal.edu

[1] Department of Biotechnology, Manipal Institute of Technology, Manipal Academy of Higher Education, Manipal 576104, India

[2] Department of Mechanical Engineering, Manipal Institute of Technology, Manipal Academy of Higher Education, Manipal 576104, India

(Pandey et al. 2001). A wide variety of agricultural residues have been previously studied in both submerged fermentation (SmF) and solid-state fermentation (SSF) processes for economic production of industrial products. Among these processes, SSF has been found to be the most suitable system for the exploitation of agricultural residues in the synthesis of industrially important enzymes (Pandey et al. 2001). The SSF process of agricultural residues brings about commercial and manufacturing advantages over SmF. These consist of a high yield of product, cost-effective upstream and downstream processes, the minimum amount of effluent generation, and lower capital investment (Pandey et al. 2000). Culture conditions such as moisture content, inoculum size, temperature, and aeration rate significantly affect the SSF process, as well as consecutively affect the fermentation products (Pandey et al. 1999). Though quite a lot of studies have been carried out using synthetic tannic acids in the production of tannase by means of SmF process (Mukesh et al. 2015; Vikas et al. 2013), whereas limited attempts have been made to produce tannase using agricultural residues as substrate in the SSF process (Natarajan and Rajendran 2012; Sabu et al. 2006). Then again, most of the research on bacterial tannase production under SSF has been carried in shake flasks, and very little study has been made in the bioreactors for the production of tannase in SSF (Mata-Gómez et al. 2015; Rodriguez-Duran et al. 2011; Sabu et al. 2006). To the best of the authors' knowledge, no reports on tannase production by *Bacillus gottheilii* M2S2 in packed bed reactors using *Triphala* as a substrate have been published. The present study reports on the bioconversion of *Triphala* a cost-effective tannin substrate for the production of tannase in laboratory-scale packed bed reactor by *B. gottheilii* M2S2. In this study, one variable at a time and response surface methodology (RSM) techniques were used to study the interaction effects of three independent variables, including the initial moisture content, inoculum volume, and the aeration rate on tannase production, and to determine the most favorable levels of these variables to achieve the maximum yield of tannase. In addition, the optimization tool, genetic algorithm (GA), was used with RSM to predict and validate the process conditions for the production of tannase in PBR. Furthermore, two types of artificial neural network (ANN) models are also implemented to predict the tannase activity for input variable values not used in the experimentation.

Materials and methods

Reagents

All the chemicals and reagents used were of analytical grade and were procured from Hi-Media and Merck.

Culture conditions and inoculum preparation

The isolated strain *B. gottheilii* M2S2 (MTCC 12554 and Accession number no. *KU866380*) from the tannery effluent soil sample as described in our previous report (Subbalaxmi and Vytla 2016) is used in this study and was maintained on nutrient agar slants and preserved at 4 °C.

The inoculum of *B. gottheilii* M2S2 was developed in a 50 mL nutrient broth and incubated at 32 °C for 20 h. It was established that the total number of viable cell count was 4×10^{12} CFU/mL by means of the colony count technique (Subbalaxmi and Vytla 2016).

Solid substrate

An inexpensive crude tannin substrate *Triphala* was obtained from the native market place in Manipal, India. The formulation of Triphala generally consists of equal proportions of pericarps of the three myrobalans: *T. bellirica, E. officinalis,* and *T. chebula* (Bali et al. 2013).

Estimation of tannins: qualitative and quantitative methods

The presence of tannins in *Triphala* was quantified qualitatively by taking a sample of 0.5 g and distilled water of 20 mL in a test tube and boiled for 10 min. Further with the Whatman filter, the mixture was filtered and to the filtrate, few drops of 0.1% $FeCl_3$ were added, and the color change was observed. Development of blue–black or brownish green color points out the existence of tannins Fig. 1 (Evans and Trease 1989).

Protein precipitation method was adopted to quantify the tannin content in crude substrate *Triphala* (Ann-Hagerman and Larry-Butler 1978). The protein bovine serum albumin

Fig. 1 A qualitative method to detect the presence of tannins in the substrate *Triphala.* Development of brownish green or blue–black coloration indicates the presence of tannins

precipitates tannins present in *Triphala* and forms tannin-protein complex. This precipitate is dissolved in sodium dodecyl sulfate-triethanolamine, and spectrophotometrically, the colored solution formed with ferric chloride reagent was measured. It was observed that the *Triphala* consisted of 7% (w/v) of tannic acid.

Moistening media

A mineral solution containing (% w/v) tannic acid, 1.9; sucrose, 0.5; NH_4NO_3, 0.5; KH_2PO_4, 0.1; $MgSO_4$, 0.1; $CaCl_2{\cdot}2H_2O$, 0.05; and NaCl, 0.1 having a pH of 5.0.

Experimental setup and solid-state fermentation

The reactor is of 20 cm height with 2.5 cm internal diameter, made up of glass, and a rubber cork with a 0.3 cm diameter hovel was placed at the bottom of the reactor to support glass beads, an air passage, and substrate *Triphala*. Further for the uniform distribution of air, the reactor was filled with glass beads up to 5 cm in length (Fig. 2). The production medium comprised of *Triphala* moistened with an optimized mineral solution and it was prepared in the reactor itself by adjusting to the required moisture content as described by Shaligram et al. (2008) and sterilized in an autoclave at 121 °C for 20 min at 15 psi. After sterilization, it was inoculated aseptically with 1 mL of inoculum with 5 g *Triphala* powder. The airflow through the reactor was the first filter sterilized using a sterile polytetrafluoroethylene membrane filter of size 0.2 µm (Millipore, USA) and its flow was controlled with the control valve. Furthermore, the filtered air was humidified by passing them to the humidification bottle and the resultant humidified air move into the PBR at the bottom and go out at the top. The PBR was kept for incubation for 32 h at 32 °C. To each experiment, the air flow rate, amount of substrate, and moisture content were adjusted to levels as shown in the experimental design matrix (Table 2). The air flow rate of various levels was set by following the principle of water displacement method as described elsewhere (Subbalaxmi and Vytla 2018; Derakhti et al. 2012; Qureshi et al. 2005):

$$\%\ \text{moisture content} = \frac{\text{inoculum volume (g)} + \text{moistening media (g)}}{\text{substrate (g)} + \text{inoculum volume (g)} + \text{moistening media (g)}} \times 100.$$

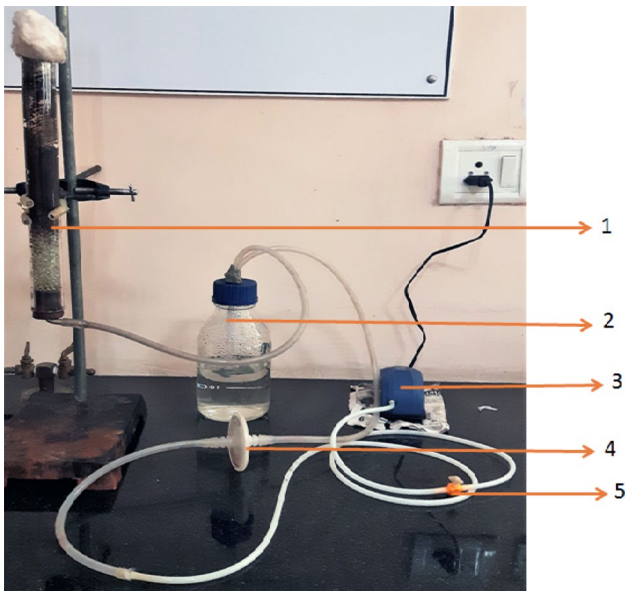

Fig. 2 Batch packed bed reactor with *Triphala*: (1) fermentation column, (2) air saturation bottle, (3) air pump, (4) air sterilization filter, and (5) control valve

Cell-free extract

After fermentation, 1 g of the fermented substrate was taken and cell-free supernatant was extracted by adding 20 mL of 0.05 M citrate buffer (pH 5.0). The flasks were then kept in a rotatory shaker for 15 min at 180 rpm and the cells were separated by cold centrifuged at 8500*g* and 4 °C for 15 min. The cell-free extract was collected in ampoules and well preserved for further examination. Experimental tests were carried out independently in duplicates and tannase activities were determined.

Tannase activity assay

The enzyme tannase was quantified using the spectrophotometric method with substrate methyl gallate as described elsewhere (Sharma et al. 2000). One unit of tannase is defined as one micromole of gallic acid formed per minute, under experimental conditions.

Experimental design

Screening of significant parameters by one variable at a time technique

Optimum physicochemical and nutrient parameters required for maximum tannase production from *B. gottheilii* M2S2 in PBR using *Triphala* as substrate were determined for the initial moisture content (40–70%), amount of substrate (5–25 g), and aeration rate (0–2 L/min). The protocol adopted for optimization of process parameters was to evaluate the effect of an individual parameter and to incorporate it at the optimized level in the experiment before optimizing the next parameter. After optimizing all parameters, a time-course experiment was conducted incorporating all the optimized parameters. All experiments were carried out in

Table 1 Experimental range and levels of the variables used in CCD in terms of coded levels and actual values for tannase production with *Triphala* from *B. gottheilii* M2S2 in PBR

Variables with designate	Coded levels				
	−2	−1	0	+1	+2
Amount of substrate (g)	5.0	10	15	20	25
Moisture content (%)	40	45	50	55	60
Aeration rate (L/min)	0.8	1.0	1.2	1.4	1.6

duplicate and the mean values were reported with standard deviation.

Optimization of critical parameters using central composite design

A 2^3 factorial central composite experimental design with four start points ($\alpha = 2$) and six replicates at the central point, resulting in 20 experiments were used to optimize the screened variables (Minitab 17.0). The screened variables with experimental range and levels were used in CCD for tannase production from *B. gottheilii* M2S2, which are shown in Table 1, and the experimental designs for tannase production using PBR are shown in Table 2.

Table 2 Experimental design matrix of CCD for tannase production with *Triphala* using *B. gottheilii* M2S2 in PBR

Run	X_1	X_2	X_3	Tannase activity (U/gds)	
				Experimental	Predicted
1	10	45	1	0.208 ± 0.082	0.199
2	20	45	1	0.101 ± 0.068	0.104
3	10	55	1	0.121 ± 0.048	0.125
4	20	55	1	0.118 ± 0.064	0.119
5	10	45	1.4	0.185 ± 0.021	0.187
6	20	45	1.4	0.051 ± 0.005	0.051
7	10	55	1.4	0.141 ± 0.023	0.141
8	20	55	1.4	0.083 ± 0.041	0.095
9	15	50	1.2	0.226 ± 0.009	0.231
10	15	50	1.2	0.226 ± 0.009	0.231
11	15	50	1.2	0.243 ± 0.025	0.231
12	15	50	1.2	0.243 ± 0.025	0.231
13	5	50	1.2	0.195 ± 0.008	0.197
14	25	50	1.2	0.061 ± 0.008	0.055
15	15	40	1.2	0.192 ± 0.022	0.19
16	15	60	1.2	0.172 ± 0.048	0.165
17	15	50	0.8	0.089 ± 0.016	0.091
18	15	50	1.6	0.060 ± 0.030	0.055
19	15	50	1.2	0.258 ± 0.031	0.263
20	15	50	1.2	0.258 ± 0.031	0.263

X_1, amount of *Triphala* (g); X_2, moisture content (%), X_3 aeration rate (L/min)

Statistical model

The linear, quadratic, and linear interactions of each parameter with their regression coefficients were fitted to a second-order polynomial equation (Eq. 1):

$$Z = \lambda_0 + \sum_{i=1}^{5} \lambda_i p_i + \sum_{i=1}^{5} \lambda_{ii} p_i^2 + \sum_{i=1}^{4} \sum_{j=i+1}^{5} \lambda_{ij} p_i p_j, \quad (1)$$

where Z is the predicted tannase activity (response); λ_0 is the intercept; λ_i and λ_{ii} are the linear and quadratic coefficients for the variable i, respectively, whereas λ_{ij} is the interaction coefficients between variables i and j. The fitness of the second-order polynomial model equation was tested based on the coefficient of determination R^2 and F test from analysis of variance (ANOVA). This model equation can be validated by carrying out the SSF process with *Triphala* in duplicates at optimal conditions in PBR and then quantifying the enzyme tannase (Subbalaxmi and Vytla 2016).

Optimization by genetic algorithm (GA)

Genetic algorithms are a type of optimization algorithm, used to find the optimal solution(s) to a given computational problem that maximizes or minimizes a particular function. GA represents one branch of the field of study called evolutionary computation in that they imitate the biological processes of reproduction and natural selection to solve for the 'fittest' solutions (Kinnear 1994; Goldberg 1989). Like in evolution, many of genetic algorithm's processes are random; however, this optimization technique allows one to set the level of randomization and the level of control (Goldberg 1989). These algorithms are far more powerful and efficient than random search and exhaustive search algorithms (Kinnear 1994); yet require no extra information about the given problem. This feature allows them to find solutions to problems that other optimization methods cannot handle due to a lack of continuity, derivatives, linearity, or other features. In this study, the second-order polynomial equation obtained from the RSM was used as the fitness function to carry out GA. For the production of tannase, the functions selected to carry out the GA are rank scaling function, constrain-dependent uniform creation function, scattered crossover function, stochastic uniform selection function, and non-linear constraint algorithm (Augmented Lagrangian). The parameters used in the computational optimization by GA were chromosome length (50), population size (50), crossover fraction (0.8), and the number of generations (100). The optimization toolbox of MATLAB R2015b was

used for the GA studies. The objective function can be given as follows:

$$\text{Maximize } z = f(y); y_i^{\mathrm{L}} \le y_i^{\mathrm{U}} \le y_i^{\mathrm{U}}, \quad i = 1, 2 \ldots n, \tag{2}$$

where $f(y)$ is the objective function obtained from the RSM studies, y is an input variable, and z represents the response obtained. The symbols y_i^{L} and y_i^{U} designate the upper and lower levels of y_i^{U}.

Artificial neural networks

Artificial neural networks (ANNs) are a class of machine learning techniques which are capable of mapping between sets of non-linear input and output variables. The use of supervised feedforward ANNs is popular in fitting an equation for the case of non-linear mapping between multiple input–output pairs. Thus, they can be used to predict the output of a system for a given set of new inputs. The ANN can be implemented in two stages—training and testing. In the training phase, the biases and the weights on the synaptic connections are initially set to very small random values. The forward pass of training essentially consists of propagating the input data for training from the input layer through the hidden layer to the output layer. The ANN outputs are obtained at the output layer, which is not the same as the desired outputs. In the backward pass of training, the training error (the difference between ANN output and desired output) is back-propagated in such a way that the synaptic weights and biases are updated (modified). One forward and backward pass constitutes an epoch. The epochs are repeated in an iterative way during which the training error continuously decreases. After the completion of a number of epochs, the training error attains a very small value, and the ANN is said to be trained. In the testing phase, the inputs not used in training are provided to the trained ANN. The corresponding ANN output obtained is found to be similar to the desired outputs (Haykin 1999; Anil 1996).

For the work carried out in this article, amounts of Triphala (X_1), moisture content (X_2), and the aeration rate (X_3) are considered as the ANN inputs, and the Tannase activity (t) acts as the output. 20 pairs of input–output values are available for training. Thus, the number of nodes in the input layer is three and that at the output layer is one. Figure 3 shows the schematic of the ANN used for modeling the Tannase production experiment. a_j ($j = 1$ to L) are the biases of the hidden layer. w_{ji} ($i = 1$ to 3; $j = 1$ to L) are the weights on the synaptic connections between the nodes of the input layer and the hidden layer. b_k is the biases of the output layer and v_{jk} are the weights on the synaptic connections between the nodes of the hidden layer and the output layer. O_1 is the output of the ANN obtained from the single node of the output layer.

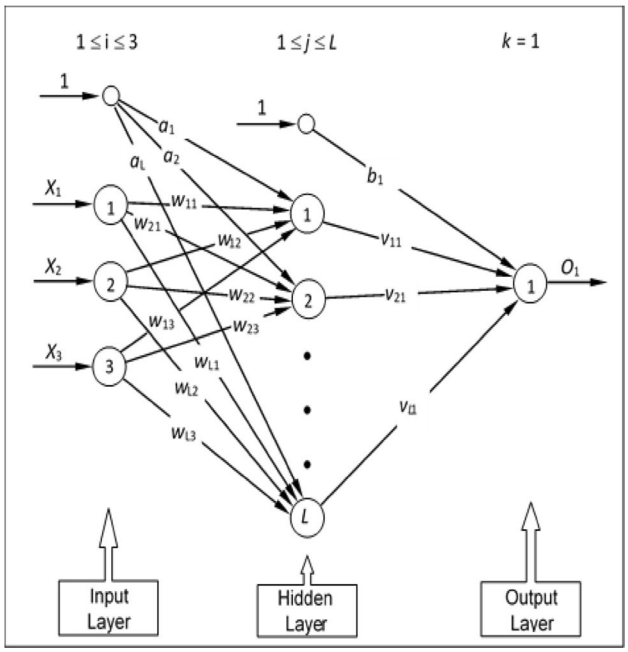

Fig. 3 Schematic of the ANN used in the work

The output of the jth node of the hidden layer is given by the following equation:

$$h_j = 1 \times a_j + \left(w_{j1}X_1 + w_{j2}X_2 + w_{j3}X_3\right). \tag{3}$$

Each hidden node output h_j is passed through a hyperbolic tangent sigmoid transfer function to get the transfer function output as y_j:

$$y_j = \frac{2}{\left(1 + \exp(-2h_j)\right)} - 1. \tag{4}$$

The output of the single node of the output layer is given as follows:

$$O_1 = 1 \times b_1 + \left(y_1 v_{11} + y_2 v_{21} + \cdots + y_L v_{L1}\right). \tag{5}$$

The performance of the ANN is measured in terms of the prediction accuracy, mean relative error, and the R^2 coefficient of regression value. These parameters are computed using the ANN output (O) and the desired output (t).

The percentage mean relative error (MRE) for training and testing are computed using the following equations:

$$\mathrm{MRE}_{\mathrm{Trg}} = \frac{100}{Q_{\mathrm{Trg}}} \sum_{i=1}^{Q_{\mathrm{Trg}}} \left(\frac{t_i - O_i}{t_i}\right) \tag{6}$$

$$\mathrm{MRE}_{\mathrm{Tst}} = \frac{100}{Q_{\mathrm{Tst}}} \sum_{i=1}^{Q_{\mathrm{Tst}}} \left(\frac{t_i - O_i}{t_i}\right). \tag{7}$$

The prediction accuracies of the ANN on the training and the test data have been computed using the following equations:

$$\eta_{\mathrm{Trg}} = \left(\frac{Q_{\mathrm{Trg}} - q_{\mathrm{Trg}}}{Q_{\mathrm{Trg}}}\right) \times 100 \tag{8}$$

$$\eta_{\mathrm{Tst}} = \left(\frac{Q_{\mathrm{Tst}} - q_{\mathrm{Tst}}}{Q_{\mathrm{Tst}}}\right) \times 100, \tag{9}$$

where q_{Trg} is the number of training pairs that are mispredicted out of a total number of Q_{Trg} training pairs, and q_{Tst} is the number of test pairs mispredicted out of a total number of Q_{Tst} test pairs. For a given pair of input–output, if the MRE between the ANN output and the desired output, for any output node is greater than ± 5, it is considered as a misprediction.

To know how best the model has fit a curve to the data under consideration, the statistical parameter R^2 given in Eq. (10) is used:

$$R^2 = \frac{\mathrm{SST} - \mathrm{SSE}}{\mathrm{SST}}, \tag{10}$$

where SST is the sum squared total and SSE is the sum squared error (Montgomery 2005).

In this work, two types of ANNs are used, viz., single hidden layer feedforward neural network (SLFNN) and the radial basis function neural network (RBFNN). The configurations of both SLFNN and RBFNN are the same, except that, in an RBFNN, the nodes of the hidden layer are replaced by RBF units. Each RBF unit is specified by its center and width (spread parameter). Both the ANNs are implemented using the MATLAB Deep Learning Toolbox (Beale et al. 2018).

Results and discussion

Screening of significant parameters by one variable at a time technique

The influence of moisture content

The maximum tannase activity of 0.176 ± 0.01 U/gds was exhibited by *B. gottheilii* M2S2 at an optimum moisture content of 50%, and whereas other parameters such as aeration rate, amount of substrate, and fermentation time were maintained at 1 L/min, 10 g, and 32 h, respectively, as shown in Fig. 4a. Away from 50% moisture content, the tannase activities were found to be decreased. The enzyme production and growth of microorganism majorly depends on the moisture content of the substrate bed (Pandey et al. 1999). Fungi require widespread series of moisture content say 20–70% to support enhanced growth and metabolism, whereas bacteria can show higher productivity simply at a

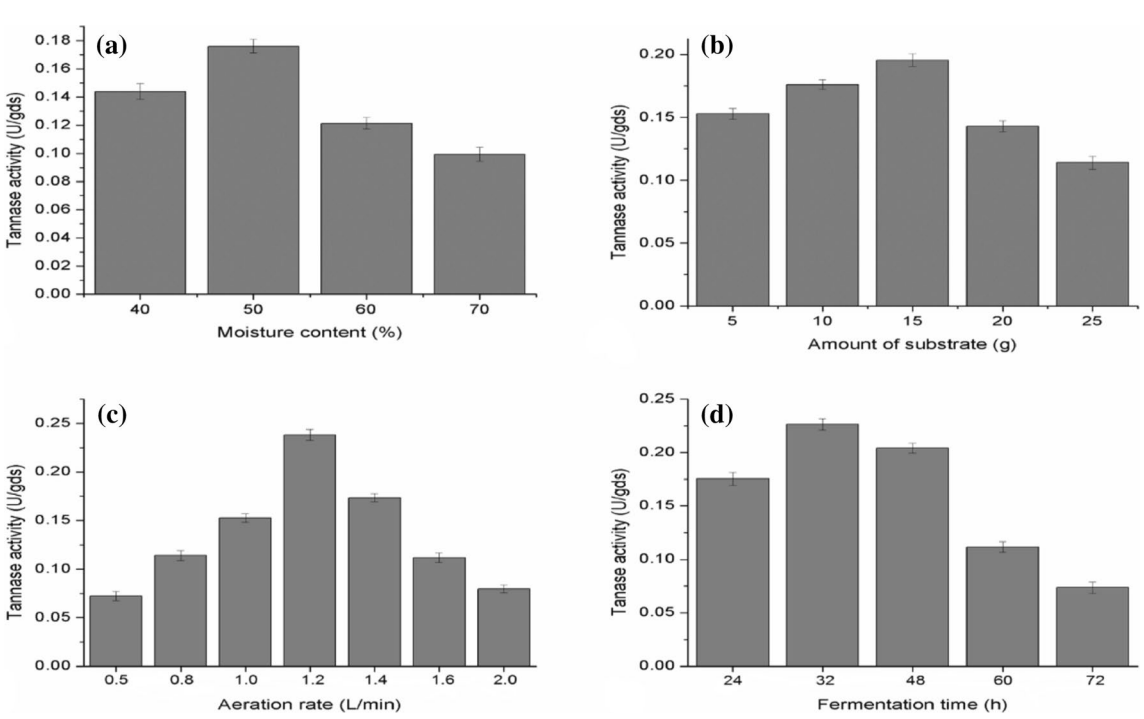

Fig. 4 Effect of process parameters on the production of tannase in packed bed reactor with PUF by *Bacillus gottheilii* M2S2. **a** Moisture content, **b** amount of substrate *Triphala*, **c** aeration rate, and **d** fermentation time

higher moisture content. However, in this study, at 60 and 70% moisture content, the tannase activities were observed to be decreased, which could be because the substrate *Triphala* was compacted and due to an external aeration of 1 L/min for the solid bed was not fixed at the place and moved towards the bottom of the packed bed reactor over the time of fermentation time. At 40% moisture content, the entire solid bed was not soaked with required mineral solution thereby showed decreased tannase activity and oxygen penetration. Hence, in PBR, maintaining an optimum moisture content plays an important role.

The influence of the amount of substrate *Triphala*

The effect of substrate *Triphala* was studied in PBR in a different range from 5 to 25 g at 1 L/min of aeration rate, 50% of the moisture content, and 32 h of fermentation time. As the amount of substrate *Triphala* increased, the tannase activity has also been increased and an optimum amount of substrate was found to be 15 g with tannase activity of 0.195 ± 0.006 U/gds (Fig. 4b). Whereas at higher substrates of 20 and 25 g, the tannase activity was found to be decreased; the reason could be that the increased bed height in the PBR led to the inadequate distribution of moistening media and air flow, thereby resulting in incomplete hydrolysis of substrate *Triphala* (Kar et al. 1999). Hence, in PBR, sustaining an optimum amount of substrate plays a significant role in the tannase production.

The influence of aeration rate

The influence of aeration rate was understood by carrying out the experiments in the different range from 0.5 to 2 L/min at 50% moisture content, 15 g substrate *Triphala,* and 32 h of fermentation time. The maximum tannase activity of 0.238 ± 0.016 U/gds was shown at 1.2 L/min (Fig. 4c), and beyond this point, there was a decline in activity which was noticed. The reason may possibly be that the air flow through the substrate bed has vaporized moisture during the course of fermentation (Derakhti et al. 2012).

The influence of fermentation time

At last, to understand the combined effect of exogenous variables such as moisture content, aeration rate, and amount of substrate *Triphala*, a time-course study was carried out at their optimum levels. The experiments were conducted including all the optimized variables. The maximum tannase activity of 0.226 ± 0.015 U/gds was exhibited at 32 h (Fig. 4d). Thereafter, the decrease in tannase activity was noticed may be due to moisture loss producing an unfavorable environment for growth and metabolism. An additional reason may possibly be the tannase which further builds glucose and gallic acid available to the microorganism. Gallic acid released over time can also inhibit the tannase action competitively, since tannic acid is composed of gallic acid units (Kumar et al. 1999; Kar et al. 1999).

Statistical optimization of significant parameters using central composite design

The significant parameters such as moisture content, aeration rate, and amount of substrate *Triphala* were further optimized using a statistical central composite design (CCD) with 20 different experimental runs for tannase production in PBR by *B. gottheilii* M2S2. The experimental design matrix with coded and real values of the above-mentioned variables is shown in Tables 1 and 2 with tannase activities. This study showed a varied range of tannase activities from 0.051 ± 0.005 U/gds (Run 6) to 0.258 ± 0.031 U/gds (Run 19) by *B. gottheilii* M2S2. The results of the experimental design matrix were fitted with a second-order and polynomial model as a function of three parameters with coded values and are shown as Eq. (1) for tannase production in PBR:

$$\begin{aligned} Y = & -2.700 + 0.00130X_1 + 0.05965X_2 + 2.608X_3 \\ & - 0.001373X_1^2 - 0.000834X_2^2 - 1.1932X_3^2 \\ & + 0.000901X_1X_2 - 0.01022X_1X_3 + 0.00728X_2X_3, \end{aligned} \quad (11)$$

where Y is tannase activity (U/gds); (X_1) amount of substrate *Triphala*, g; (X_2) moisture content, %; (X_3) aeration rate, L/min.

The results of the statistical analysis of variance (ANOVA) obtained in the present study for the production of tannase from *B. gottheilii* M2S2 with *Triphala* are shown in Table 3. The results are in good agreement with the general facts of higher F value, predicted ^{2} values, and lower PRESS values which specify a better fit. P values < 0.05 indicate that the model terms were significant. In this study, all the linear, square and interactive terms of X_1, X_2, and X_3 were significant for tannase production from *B. gottheilii* M2S2 in PBR (Table 3).

The three-dimensional surface plots have been used to visualize the interaction effects among individual parameter on tannase production by *B. gottheilii* M2S2 (Fig. 5). The surface plot of the parameters such as the amount of substrate *Triphala*, moisture content, and aeration rate indicated the prominent interaction and as maximum tannase activities at their hold values. The same phenomena are numerically shown in Table 3 (< 0.05: the presence of interaction and > 0.05: no interaction). The regression model was solved for maximum tannase production using the response

Table 3 Analysis of variance values for the quadratic regression model obtained from CCD employed in the optimization of medium for tannase production in PBR with *Triphala* from *B. gottheilii* M2S2

Source	*df*	Sum of squares	Contribution %	Mean square	*F* value	*P* value
Model	10	0.095884	99.18	0.009588	109.24	0.00*
Block	1	0.000011	0.01	0.04686	53.39	0.00*
Linear	3	0.022512	23.29	0.007504	85.5	0.00*
X_1	1	0.020264	20.29	0.020264	230.87	0.00*
X_2	1	0.000927	0.96	0.00927	10.56	0.01*
X_3	1	0.001321	1.37	0.001321	15.05	0.004*
Square	3	0.068042	70.38	0.22681	258.41	0.00*
X_1^2	1	0.01133	11.72	0.028284	322.25	0.00*
X_2^2	1	0.002038	2.11	0.010438	118.92	0.00*
X_3^2	1	0.054674	56.56	0.054674	622.92	0.00*
Interaction	3	0.005319	5.50	0.001773	20.20	0.00*
X_1X_2	1	0.004059	4.20	0.004059	46.25	0.00*
X_1X_3	1	0.000836	0.87	0.000836	9.53	0.013*
X_2X_3	1	0.000423	0.44	0.000423	4.82	0.056NS
Lack of fit	5	0.000790	0.82	0.000088	1.39	0.387NS
Error	9	0.000501	0.52	0.0001		
Pure error	4	0.000289	0.3	0.000072		
Total	19	0.96674	100			

X_1, amount of substrate, X_2, moisture content; X_3 aeration rate

$S = 0.00937$; $R^2 = 99.18\%$; adjusted $R^2 = 98.27\%$; PRESS = 0.004732; predicted $R^2 = 95.10\%$

*Statistically significant (95% confidence interval), NS, statistically not significant (95% confidence interval)

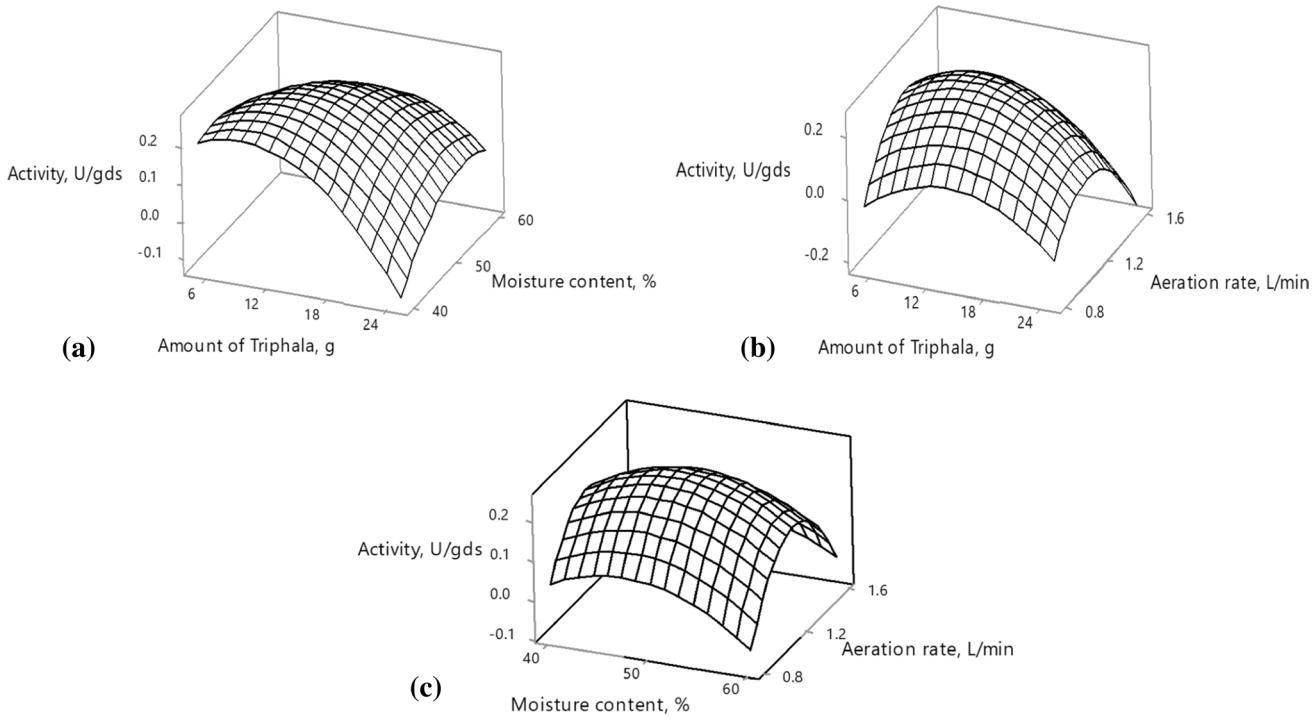

Fig. 5 Surface plots showing the interaction effects of variables on tannase production by *B. gottheilii* M2S2 with *Triphala* in PBR with the remaining factors held constant at the middle level of the CCD. **a** Amount of *Triphala* and moisture content, **b** amount of *Triphala* and aeration rate, and **c** moisture content and aeration rate

optimizer tool in MINITAB 17.0 and the optimal levels of individual parameter in real units were as follows: amount of substrate *Triphala* = 11.465 g, moisture content = 47.071%, and aeration rate = 1.188 L/min; all of them were found within the experimental levels. The predicted tannase activity under these optimal environments was 0.262 U/gds.

To validate the results, experiments were done in duplicates at the optimized values as mentioned above. Under these optimized conditions, 0.262 U/gds and 0.265 U/gds were the predicted and experimental values of tannase activities, respectively. The good correlation between the predicted observed values approves the competence of the model. This two-step optimization approach led to the improvement in tannase production by *B. gottheilii* M2S2 from 0.029 U/gds (unoptimized medium, flask scale) to 0.265 U/gds (optimized medium, PBR), an 11-fold increase. This value is comparatively higher than reported for different bacterial strains (Subbalaxmi and Vytla 2017a, b; Prasanna et al. 2009, 2010, 2012).

To the best of our knowledge, no report on bacterial tannase production in packed bed reactors using *Triphala* as crude tannin substrate under SSF has been published. Several researchers have used the laboratory-scale packed bed reactors to study the production of various industrial enzymes. Couto et al. (2000) reported the production of ligninolytic enzyme and decolorization of dye Poly R-478 from *Phanerochaete chrysosporium* BKM-F-1767 in packed bed reactor with PUF. Aeration was supplied to the reactor at 0.5 vvm and exhibited maximum lignin peroxidase of 197 U/L on the 7th day of fermentation and whereas 30% biological degradation of dye was observed. Abdeshahian et al. (2010a, b) reported β-mannanase and β-glucosidase production in a glass column reactor with palm kernel cake as a substrate from *Aspergillus niger* FTCC 5003. They used central composite design to optimize the culture conditions, and therefore, maximum β-mannanase and β-glucosidase activities of 2117.89 U/g and 52.06 U/g were obtained, respectively. Moreira et al. (1997) reported the continuous production of manganese peroxidase production in a packed bed reactor with PUF from *P. chrysosporium.* Variables such as nutrient feed rates, manganese concentration, use of air or oxygen, hydraulic retention time, and recycling flow were optimized for production of manganese peroxidase and thereby showed the maximum activity of 250 U/L. The success of the production process in a packed bed reactor majorly depends upon the correct choice of the solid medium. However, very limited studies have been carried out with packed bed reactor for the production of tannase and were predominantly from fungal source with inert support PUF (Mata-Gómez et al. 2015; Rodriguez-Duran et al. 2011; Van de Lagemaat and Pyle 2001, 2004).

Optimization and validation based on GA

GA produces global results, whereas local results by RSM. To get the global results, optimization based on GA was repeatedly carried out numerous times for accuracy. The optimal conditions identified by GA based on RSM was found to be 11.532 g of substrate *Triphala*, 47.071% moisture content, and 1.188 L/min aeration rate. The tannase yield (U/g) under optimized conditions determined by GA based on RSM was 0.263 U/g, which was almost similar to 0.262 which was predicted by RSM under optimal conditions. The optimal results obtained by both GA and RSM were found to be comparable, and hence, the model

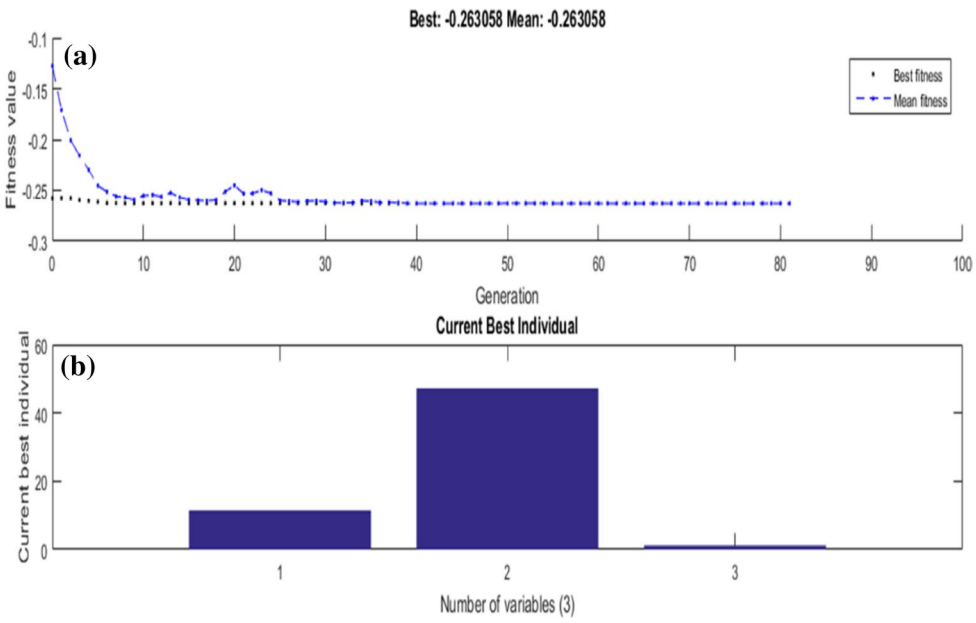

Fig. 6 Plots of genetic algorithm: **a** best fitness and **b** best individual

proposed (Eq. 11) can be used to predict the tannase production for given conditions. The best fitness value and the corresponding best individual are shown in Fig. 6. The negative sign in the best fitness plot was because of the inclusion of a negative sign on the regression equation. Moisture content was found to be the best individual in GA. To confirm the predicted result, a validation experiment was carried out under optimized conditions predicted by RSM-GA model. It exhibited a tannase activity of 0.265 U/g.

Comparison of the RSM and ANN models

Table 4 shows the values of the tannase production obtained from an experiment in the third column and the values predicted by the RSM model, SLFNN model, and the RBFNN model, respectively, in the fourth, fifth, and sixth columns. It can be observed that the prediction of the ANN models is relatively closer to the experimental values. However, the prediction performances of the three models can be statistically compared by computing their prediction performance parameters given in Eqs. (6), (7), (8), (9), and (10). The computed values are shown in Table 5.

Table 5 Comparison parameter values for RSM model, SLFNN, and RBFNN

	Models		
	RSM	SLFNN	RBFNN
Prediction performance parameters			
MRE_{Trg}	3.586%	1.357%	1.357%
MRE_{Tst}	25.719%	13.559%	13.707%
η_{Trg}	85%	80% ($^{a}L=90$)	80% ($^{a}\sigma=0.411$)
η_{Tst}	0	40%	20%
R^2	0.9864	0.9930	0.9949

[a]L, no. of hidden neurons in SLFNN, σ, spread parameter in RBFNN

It is clear from Table 5 that the prediction performance of the SLFNN is superior to those of RBFNN and RSM. It can also be seen that the RBFNN performs better than the RSM model. Figure 7a–c shows the residual chart for the RSM, RBFNN, and SLFNN models, respectively. Again,

Table 4 Tannase production experiment results and predicted values using RSM, SLFNN, and RFFNN

Expt. no.	Experimental	RSM predicted	SLFNN predicted	RBFNN predicted
Training				
1	0.208 ± 0.082	0.199	0.208	0.208
2	0.101 ± 0.068	0.104	0.101	0.101
3	0.121 ± 0.048	0.125	0.121	0.121
4	0.118 ± 0.064	0.119	0.118	0.118
5	0.185 ± 0.021	0.187	0.185	0.185
6	0.051 ± 0.005	0.051	0.051	0.051
7	0.141 ± 0.023	0.141	0.141	0.141
8	0.083 ± 0.041	0.095	0.083	0.083
9	0.226 ± 0.009	0.231	0.2423	0.2423
10	0.226 ± 0.009	0.231	0.2423	0.2423
11	0.243 ± 0.025	0.231	0.2423	0.2423
12	0.243 ± 0.025	0.231	0.2423	0.2423
13	0.195 ± 0.008	0.197	0.195	0.195
14	0.061 ± 0.008	0.055	0.061	0.061
15	0.192 ± 0.022	0.19	0.192	0.192
16	0.172 ± 0.048	0.165	0.172	0.172
17	0.089 ± 0.016	0.091	0.089	0.089
18	0.060 ± 0.030	0.055	0.06	0.06
19	0.258 ± 0.031	0.263	0.2423	0.2423
20	0.258 ± 0.031	0.263	0.2423	0.2423
Testing				
21	0.121 ± 0.009	0.0423	0.1202	0.0978
22	0.144 ± 0.0075	0.1915	0.1696	0.1862
23	0.196 ± 0.006	0.2093	0.1628	0.1848
24	0.176 ± 0.009	0.2003	0.1681	0.1836
25	0.174 ± 0.009	0.1913	0.2227	0.1566

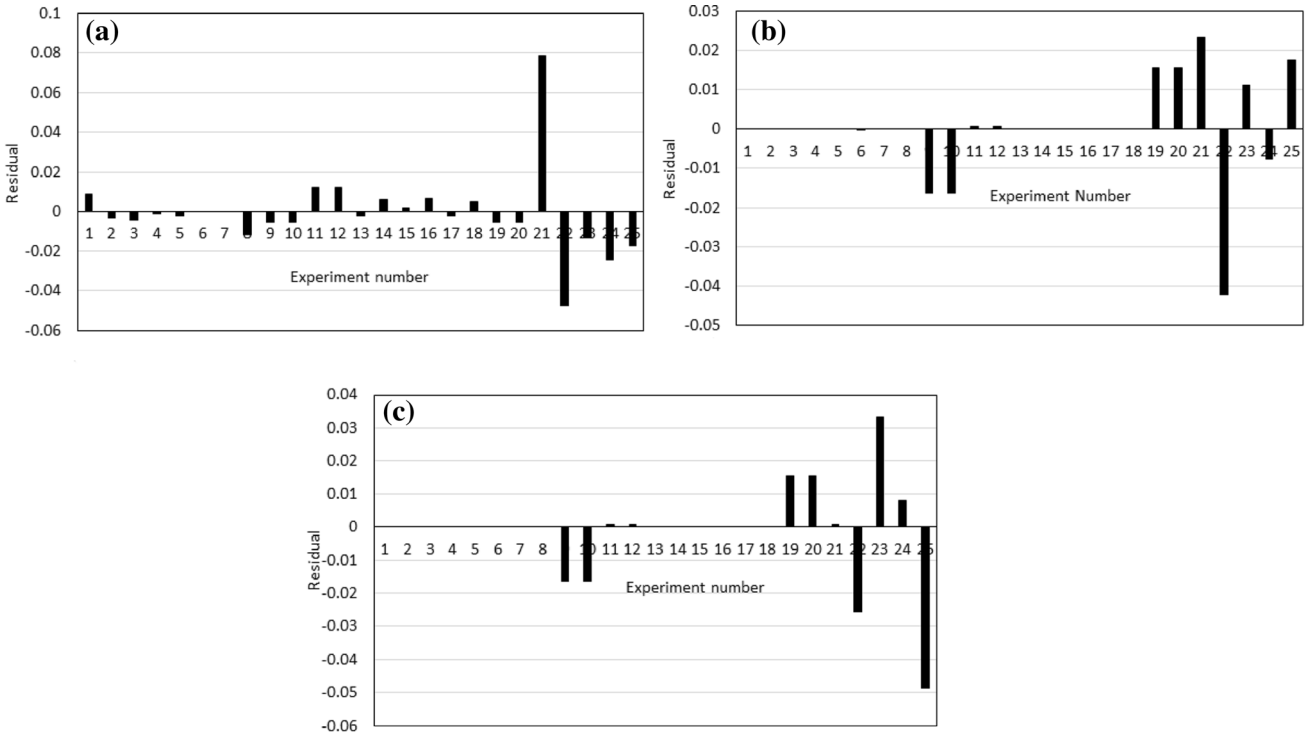

Fig. 7 Residual chart for the RSM model (**a**); residual chart for the model RBFNN (**b**) and residual chart for the SLFNN model (**c**)

it is clear from the charts that the SLFNN model has the least residuals.

Conclusions

The results confirmed the inexpensive agricultural residue *Triphala* as a prominent substrate for production of tannase. The optimized and validated models had good agreement with each other. The critical values of process parameters predicted by RSM and GA were found to be 11.532 g of substrate *Triphala*, 47.071% moisture content, and 1.188 L/min aeration rate with highest tannase activity of 0.265 U/gds. In addition, a comparison of the prediction performances of the RSM and the ANN models SLFNN and RBFNN reveals that the ANN models ($R^2 = 0.9949$) are better predictors than the RSM ($R^2 = 0.9864$), and, furthermore, to understand the behavior of the strain, need to carry out the kinetic studies, and develop a suitable kinetic model.

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Compliance with ethical standards

Conflict of interest The authors certify that no actual or potential conflicts of interest in relation to this article exist.

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