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MACHINE LEARNING APPLICATION FOR OPTIMIZING ASYMMETRICAL REDUCTION OF ACETOPHENONE EMPLOYING COMPLETE CELL OF *LACTOBACILLUS SENMAIZUKE* AS AN ENVIRONMENTALLY FRIENDLY APPROACH

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ABSTRACT

Recently, optimization of the bioreduction reactions by optimization methodologies has gained special interest as these reactions are affected by several extrinsic factors that should be optimized for higher yields. An important example for these kinds of reactions is the complete cell implications for the bioreduction of prochiral ketones in which the culture parameters play crucial roles. Such biocatalysts provide environmentally friendly and clean methodology to perform reactions under mild conditions with high conversion rates. In the present work, at the first step the *Lactobacillus senmaizuke* was isolated from sourdough and the complete cell application of *Lactobacillus senmaizuke* for the bioreduction of acetophenone was optimized by an Artificial Neural networks (ANNs) to achieve the highest enantiomeric excess (*EE*, %). The culture parameters, pH, temperature, incubation period and agitation speed were the experimental factors that were optimized to maximize *EE* (%) by machine learning algorithm of Artificial Intelligence modeling and the best conditions to maximize *EE* (95.5 %) were calculated to be pH of 5.7, temperature of 35 ºC, incubation period of 76 h and agitation speed of 240 rpm with very low sum of squared error value (0.611236 %) to bioreduce acetophenone using complete cell of *Lactobacillus senmaizuke* as a sourdough isolate GRAS microbial species. Accordingly, The ANN was employed to correctly establish the enantiomeric excess values of the specimen with an average absolute error 0.080739 %.

Keywords: Sourdough, Asymmetric bioreduction, Biocatalyst, Chirality, Machine learning, ANNs, Biotransformation

INTRODUCTION

The asymmetric nature of biological macromolecules reveals the importance of chirality for life, technology and chemistry to obtain the desired molecular chiral structures using different techniques. In organic chemistry, production of enantioselective prochiral ketones is a vital process and using biocatalysts is method of choice compared to the chemical synthesis due to important advantages of the process including environmentally friendly and cheap reaction conditions. Importantly, enantiomerically pure secondary chiral alcohols are crucial elements with respect to synthesize distinct substances used in different industries such as pharmaceutical and agriculture [1-5]. There are several examples of drugs produced from these molecules such as Duloxetine and Ezetimibe that are used for the treatments of affective disorder, generalized anxiety disorder, fibromyalgia, neuropathic pain and lowering plasma cholesterol levels. [6,7] Another example of drug that chiral secondary alcohols play roles for its biosynthesis is isoprenaline which is utilized to treat bradycardia. [8]

There is a growing sales level for the pharmaceutical products with single enantiomeric form increasing yearly. This results in the increment in the attempts to explore novel enantioselective routes to acquire single enantiomer of substances. Both agriculture and food industries are positively affected from these attempts as these molecules are also important for the mentioned industries. The attempts to find new routes to produce enantioselective chiral compounds are targeting to produce these molecules more efficiently and more selectively than the current methodologies [9-12]. One of these attempts is the use of biocatalysts to produce enantiopure alcohols from prochiral ketones in a simple, mild, cost-effective, and ecofriendly conditions. For instance, several reports revealed the fact that ketoreductases were excellent biocatalysts to produce enantiopure secondary alcohols using prochiral ketones [13-17]. Chemical catalysts can also be used for the production of enantiopure secondary alcohols but they have important disadvantages compared to biocatalysts such as their cost levels, low conversion and poor enantioselectivity rates and importantly requirement for the harsh circumstances that might result in the formation of toxic and undesirable compounds. In comparison to the chemical catalysts, biocatalysts provide environmentally friendly and clean methodology to perform reactions under mild conditions with high conversion rates. In terms of green technology, use of biocatalysts is an important methodology in order to produce these crucial enantiopure secondary alcohols under mild reaction conditions with no detrimental effects to the environment. Two types of biocatalytic operations can be industrially applied as asymmetric reduction of prochiral ketones or the resolution of racemate. In the former application, the enantiomer can be produced with high conversion yields and enantiomeric purity. The biocatalysts can be applied as whole cells or purified enzymes to proceed the bioreduction reactions and whole cell application has some advantages compared to the purified enzymes such as the presence of the cofactors in the cytoplasm of the cell which should be added in the enzyme application and low level of costs compared to the purified enzymes [9-12]. It should be also noted that enzymes have important level of increased activity rates and stability. With the help of improvements in biotechnological approaches, more chemical compounds will be subjected to the bioreduction reaction to produce these compounds from inexpensive basic materials under environmentally-kind mild operations. [18] Microorganisms are one of the main examples of biocatalysts and whole cell yeast and bacteria applications were successfully shown to perform the asymmetric reduction of the carbonyl moiety to chiral alcohols. As mentioned above the extrinsic parameters play crucial roles for the asymmetric reduction reactions and examples of these parameters are incubation temperature, incubation pH, incubation period and the agitation level during the reactions. [19] These parameters can be tested individually to explore their effects for the final enantiomeric excess levels, but it is highly time consuming and very expensive to test these parameters individually in which the results of each parameter cannot be linked easily. Therefore, to solve these problems and better understand their interactions, several optimization techniques became the method of choice of some computational methods. In this regard, some multivariate statistical analyses such as a machine learning approach ANs and relevant chemometrics in general are useful computational tools although they follow fully different research approaches. Besides, increasingly applied some computational methods can be used to model biocatalysis reactions by orienting experimental planning, consequently modulating; for example, the enzyme activity to change microbial metabolic reactions. [20] Therefore, in this study, ANN's optimization methods were used to optimize more

easily and efficiently asymmetrical reduction of acetophenone by employing complete cell of *Lactobacillus senmaizuke* as a biocatalyst in asymmetric reduction of acetophenone to 1-phenyl ethanol.

MATERIALS AND METHODS

General

The chemicals and solvents used in this study were from Sigma-Aldrich (purity of >99%) except the bacterial medium (MRS) which was purchased from Merck. Thin-layer chromatography (TLC) was used to visualize the reaction products in TLC plates (aluminum, silica gel 60 F254 Merck, 0.25 mm) and the developing solution was hexane: ethyl acetate (4:1, v/v). Column chromatography was used to purify (R)-1-phenylethanol using hexane: ethyl acetate (10:1, v/v) as elution solution. High-performance liquid chromatography (HPLC) analysis was performed on an Agilent 1260 systems equipped with a UV and chiral detector. The racemic 2 was obtained by reducing the 1 with NaBH4 in methanol at room temperature used as a standard for the determination of the (R)- or (S)-enantiomers. Optical rotation was measured with a Bellingham + Stanley, ADP220, 589 nm spectropolarimeter. 1H and 13C NMR spectra were recorded on Bruker 400 MHz spectrometer in CDCl3. The conversion was determined by chromatography on a chiral column on HPLC after filtering the crude product with a column containing small silica gel and comparing the alcohol peaks with the ketone peak. Enantiomeric excess was also determined by HPLC with the chiral column. 1H- and 13C-NMR, and HPLC spectra can be found in the Supporting Information. (R)-1-phenylethanol (2): Colorless oil, Yield 90%, 1H NMR (400 MHz, CDCl3) δ= 7.38-7.33 (m, 4H, Ar), 7.31-7.26 (m, 1H, Ar), 4.84 (q, J = 6.45 Hz, 1H, CH), 2.62 (bs, 1H, OH), 1.48 (d, J = 6.5 Hz, 3H, Me); 13C NMR (100 MHz, CDCl3) δ= 146.0 (ArC), 128.4 (ArC), 127.3 (ArC), 125.5 (ArC), 70.2 (CCH), 25.2 (CMe); [α]D 25 = +69.7 (c 1.1, CHCI3), 80% ee; HPLC (Chiralcel OD-H column, n-hexane/i-PrOH, 95:5, flow rate of 1.0 mL/min, 210 nm) tR (R) 8.8 min, (S) 10.3 min [21]. The HPLC condition of 1: Chiralcel OD-H column, hexane/i-PrOH, 95:5, flow rate of 1.0 mL/min, 210 nm, 5.9 min (Supporting Information).

Experimental design and general bioreduction reactions

The Enantiomeric excess (%) (*EE*) values of the study conducted by Colak et al. [21] reported the optimization strategy for asymmetric bioreduction of acetophenone using whole cell of *Lactobacillus senmaizukei* by Response surface methodology (RSM). The data set were analyzed and optimized using machine-learning algorithm of Artificial Intelligence modeling procedures. The *EE* values describes the purity used for chiral substances and highest *EE* values reflects the formation of one type of enantiomer from the reaction. In the aforementioned study, whole cell of *Lactobacillus senmaizukei* was used for the bioreduction of acetophenone and the *EE* values were obtained and then used as the template to apply the optimization of the reaction by machine learning approach in the present study.

The optimized reaction conditions were also tested to investigate the actual *EE* values for the bioreduction of acetophenone. For this purpose, *Lactobacillus senmaizukei* was inoculated to 10 ml MRS broth and incubated 2 days at 37 °C followed by the inoculation of grown cells at 10% concentration to 50 ml MRS broth with a pH of 4.79. Following the incubation under agitation for 2 h, 0.5 mmol substrate was added to the MRS and the incubation was conducted under agitation (150 rpm) at 25 °C for 70 h. After the incubation, the supernatant was obtained and saturated with NaCl, then extracted with diethyl ether. The diethyl ether extracts were combined and dried over Na_2SO_4. After removal of the solvent under reduced pressure, the crude product was identified by NMR analysis. The absolute configuration was determined by sign of specific rotation and comparison with the literature. The enantiomeric excess of the seconder alcohol was determined by chiral HPLC analysis.

In this study, whole cells of *Lactobacillus senmaizukei* were used as a bioreduction agent that was previously isolated from sourdough. For this, serial dilutions were prepared from the traditional sourdoughs and were plated to MRS agar plates that were incubated at 37°C for 48 h and random colonies were selected from agar plates which were then subjected to 16S rRNA gene amplification by PCR analysis and this strain was identified as described previously[22].

Statistical analysis and machine learning

Machine learning is one of the sub-disciplines of Artificial Intelligence for using data available in particular environment to predict outcomes identifying (discovering) patterns in data and decision making. It describes the

process of how computers are 'learning' through human input (experimental data) and training them to obtain certain outcomes by ANNs. ANNs are modelling and computing systems capable of deep learning and composed of a set of basic high-degree interconnecting elements for data handling. In the present work, the back-propagation multilayer perceptron (BPMLP) algorithm was employed for estimating the levels of culture (input) parameters that would maximize the *EE*. The BPMLP performs a particular non-linear mapping, which could be stated with respect to a known group of input parameters (Table 1) obtained from experimental measurements such as pH (3.5–7.5, x_1), temperature (20–40 °C, x_2), incubation period (0–96 h, x_3) and agitation speed (50–250 rpm, x_4), the output parameter is the enantiomeric excess (*EE*, %, y). The BPMLP algorithm employs the steepest descent algorithm for minimization of the mean squared error of given data. It also employs the Levenberg-Marquardt (LM) approach as an optimization method to solve problems on non-linear least squares. The learning performance of this algorithm is based on adapting all synaptic weights in that the incompatibility amidst the actual output data of *EE* (%) and the targeted data (the outcomes of ANNs) might be as low as possible as their average is taken for over all the learning samples. The following steps were used for the training process of LM algorithm. Initially, randomly generated initial weights were uploaded in the ANNs to estimate the total error (SSE) of the networks.
If the initial training process is not successful enough, the weights are updated using Eq. (8).

Table 1. Data set used for ANN algorithm and the outcomes [a]

	Experimental factors				Response	Machine learning		
Runs	pH, (x_1)	Temperature (ºC) (x_2)	Incubation period (h) (x_3)	Agitation speed (rpm) (x_4)	Enantiomeric Excess (%) (y)	ANN outcomes	ANN Error	ANN Error (%)
1	4.5	35	72	200	45	44.9311	0.0689	0.02845
2	6.5	25	72	200	47	46.8127	0.1873	0.07733
3	5.5	30	48	150	56	55.9895	0.0105	0.00433
4	7.5	30	48	150	64	64.0106	-0.0106	-0.00440
5	5.5	30	48	150	56	55.9895	0.0105	0.00433
6	6.5	25	24	200	64	64.0000	0.0000	0.00000
7	4.5	35	72	100	56	56.0167	-0.0167	-0.00690
8	5.5	30	96	150	51	51.2961	-0.2961	-0.12220
9	6.5	35	24	200	27	27.0052	-0.0052	-0.00210
10	5.5	40	48	150	19	19.0359	-0.0359	-0.01480
11	4.5	25	24	200	48	47.9906	0.0094	0.00388
12	4.5	35	24	200	83	83.1334	-0.1334	-0.05510
13	5.5	30	48	50	68	68.0123	-0.0123	-0.00510
14	5.5	30	48	150	56	55.9895	0.0105	0.00433
15	5.5	20	48	150	66	66.2651	-0.2651	-0.10940
16	3.5	30	48	150	50	49.9809	0.0191	0.00789
17	6.5	35	24	100	70	70.4139	-0.4139	-0.17090
18	6.5	35	72	100	52	51.9914	0.0086	0.00355
19	5.5	30	48	150	56	55.9895	0.0105	0.00433
20	5.5	30	48	150	56	55.9895	0.0105	0.00433

21	4.5	25	72	100	94	93.8474	0.1526	0.06300
22	5.5	30	48	250	92	91.9938	0.0062	0.00256
23	4.5	25	72	200	48	48.0072	-0.0072	-0.00300
24	5.5	30	48	150	56	55.9895	0.0105	0.00433
25	6.5	25	24	100	59	59.0314	-0.0314	-0.01300
26	5.5	30	0	150	0.5	0.50016	-0.0002	-7E-05
27	6.5	35	72	200	22	22.2461	-0.2461	-0.10160
28	6.5	25	72	100	55	54.6342	0.3658	0.15102
29	4.5	25	24	100	71	70.9787	0.0213	0.00879
30	4.5	35	24	100	56	55.9541	0.0459	0.01895

The experimental data whose mean values were previously reported in the previous study (Colak et al., 2019) were used to perform machine modeling algorithm in this study.

Then the total error is evaluated using the new weights, if the present total error is augmented in consequence of the updated data, then the combination coefficient δ is increased by a factor. If the outcomes are not satisfying, then the initial step is reacted, and an update was tried again.
If the present total error is reduced by virtue of the update, in this case admit the step (for example, sustain the new weight vector as the present one) and reduce the combination coefficient δ by a factor of 10 or by the same factor as in the following step.
Go to the second step with the new weights by the time the present total error is lower than the targeted value.
In this study, we have obtained the following results for the training process of ten iterations. Figure 1 (a) shows the weight distribution allocated for obtaining the minimum error. Figure 1 (b) shows the self organizing Map (SOM), which is a learning algorithm depicting the input data of ANN used for visualization and analysis of enantiomeric excess. SOM is a topology maintaining technique to keep the neighborhood relations in its mapping presentation. Random distribution of weights causes much iteration for achieving the best learning performance; however, SOM can arrive at a map of stable zones for training of ANNs easily, and interpretation of data can be done by human. SOM is still a great machine learning technique to present the invisible patterns in the data. It constitutes a lexical chart where counterpart examples can be mapped close together and different ones apart.

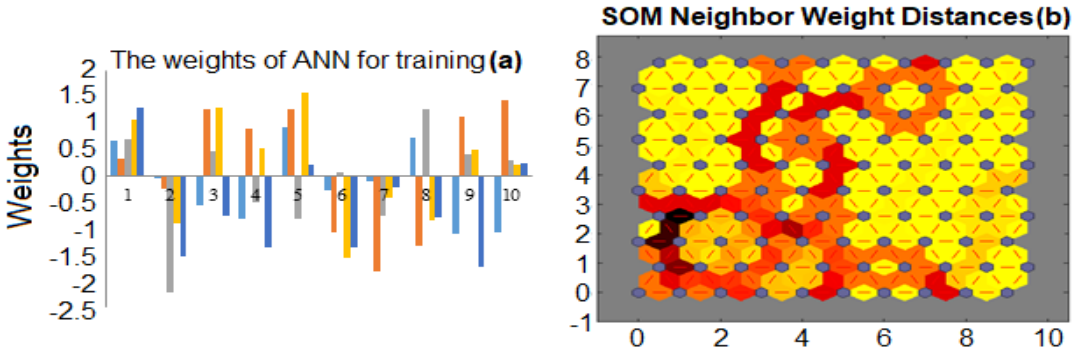

Figure 1. Synaptic weights of ANN for training process

On the other hand, the LM algorithm ensures a numerical solution to the problem of minimization of the non-linear relations. The steepest descent algorithm goes by the name of the error Backpropagation (EBP) Algorithm and considered as one of the most important advancements for the training of ANNs. However, the disadvantage of this algorithm is the slow convergence which could be remarkably enhanced by the Gauss–Newton algorithm. It is

possible for Gauss–Newton algorithm to get suitable step magnitudes for every aspect and converge too rapid when the second-order derivatives of error function is employed to analyse the curvature of error surface. When the error function has a quadratic surface, the algorithm is able to converge easily and directly after some iteration (see Figure 2). Two minimization methods are combined by LM algorithm; the Steepest Descent method and the Gauss–Newton algorithm to fit the error curve. These parameters are updated in the Steepest Descent direction, which makes this combination to reduce the sum of the squared errors. Figure 2 depicts the sum of the squared errors decreased by supposing the least squares function, being locally quadratic to find the minimum of the error.

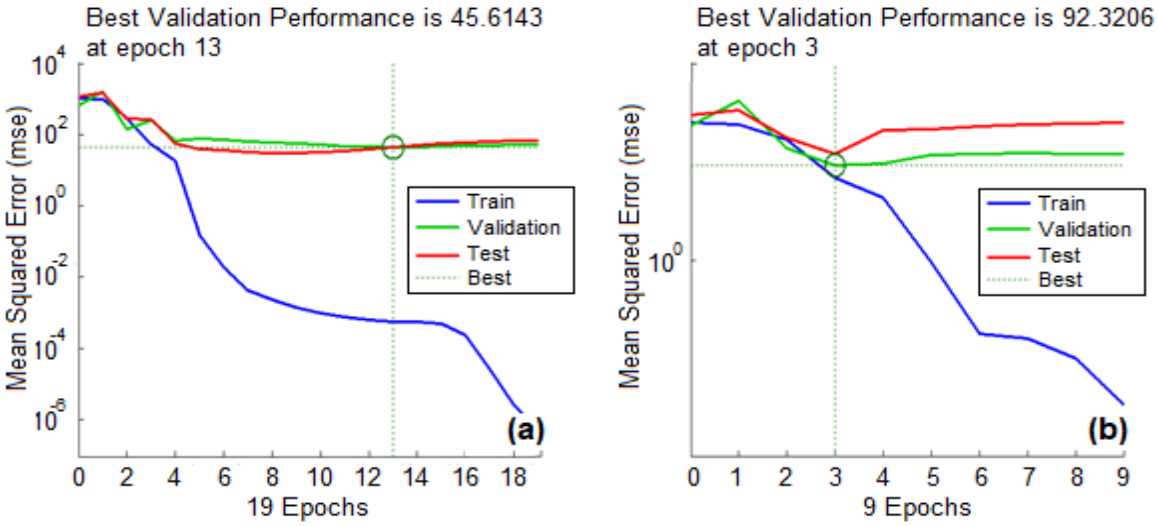

Figure 2. The types of errors occurred for the initial (a) and final (b) training of ANNs

Steepest descent algorithm

The first-order algorithm is also known as the steepest descent algorithm, which employs the first-order derivative of total error function in order to calculate the minimum in error space. [23] Generally, gradient g is described as the first-order derivative of total error function (see Eq. (1)):

$$g = \frac{\partial E(x,w)}{\partial w} = \left[\frac{\partial E}{\partial w_1} \quad \frac{\partial E}{\partial w_2} \quad \cdots \quad \frac{\partial E}{\partial w_N} \right]^T \tag{1}$$

With the gradient g, the update rule of the steepest descent algorithm can be expressed as given in Eq. (2).

$$w_{k+1} = w_k - \alpha g_k \tag{2}$$

where α is the learning constant. The steepest descent algorithm has the training process which is a asymptotic convergence. For solution, all the elements of gradient vector will be tiny and weight change will be very small.

Gauss Newton algorithm

When Newton's method is implicated to update weight to obtain the Hessian matrix (H), the second-order derivatives of total error function is computed in too complicated way. For simplification of the computing process, a Jacobian matrix J could be utilized. As Gauss -Newton method assumes that all the gradient components g_1, g_2,…, g_N are functions of weights and all weights are linearly independent, the elements of gradient vector could be computed as given in Eq.(3).

$$g = Je \tag{3}$$

where error vector e has the form, $e_{1,1,}$ $e_{1,2}$,…,$e_{p,M}$. On the other hand, the element at ith row and jth column of Hessian matrix could be computed as given in Eq.(4).

$$h_{i,j} = \sum_{p=1}^{p} \sum_{m=1}^{M} \frac{\partial e_{p,m}}{\partial w_i} \frac{\partial e_{p,m}}{\partial wi} + S_{i,j} \tag{4}$$

The error function ($S_{i,j}$) is close to zero in Eq. (3), hence the link amidst Hessian matrix H and Jacobian matrix J could be retyped as it is given in Eq. (5).

$$H=J^T J \tag{5}$$

When Newton's equations and elements of gradient vector are combined, the update rule of the Gauss–Newton algorithm can be given as shown in Eq. (6).

$$w_{k+1} = w_k - \left(J_k^T J_k \right)^{-1} J_k e_k \tag{6}$$

The obvious benefit of the Gauss–Newton algorithm is that the computation of the second-order derivatives of the total error function is not required, by employing the Jacobian matrix J.

Levenberg –Marquardt (LM) Algorithm

Since the Hessian matrix $J^T J$ is invertible, LM algorithm offers alternative approach to Hessian matrix presented in Eq. (7).

$$H \approx J^T J + \delta I \tag{7}$$

where, δ is a positive combination coefficient, I is the identity matrix from Eq. (7), in which the elements of the Hessian matrix will be bigger than zero and is always invertible. If the Eq. 6 and Eq. (7) are combined, the updated rule of LM algorithm could be given as in Eq. (8).

$$w_{k+1} = w_k - \left(J_k^T J_k + \delta I \right)^{-1} J_k e_k \tag{8}$$

As LM algorithm combines the steepest descent algorithm and the Gauss–Newton algorithm, it switches between the two algorithms during the training process and gains the advantages of both. Selecting a very small (nearly zero) combination coefficient δ, Eq. (8) will approach to the Eq. (6) and Gauss–Newton algorithm will be employed. Conversely, if combination coefficient δ is selected very large, Eq. (8) will approximate to Eq. (7) and the steepest descent method will be employed. A large combination coefficient δ in Eq. (8), is inferred as the learning coefficient in the steepest descent method. [24, 25] The learning coefficient is $\alpha = 1/\delta$ the inverse of combination coefficient.

Implementing the LM algorithm for training of pH (3.5–7.5), temperature (20–40 °C), incubation period (0–96 h) and agitation speed (50–250 rpm), and the output parameter enantiomeric excess (*EE*, %) with ANNs, two problems have to be solved; the calculation of the Jacobian matrix, and organization of the training process iteratively for the weight updating. Considering a neuron j with n_i, inputs, as presented in Figure 3, neuron j is in the first layer, all its inputs are connected to the inputs of the network. On the other hand, its inputs are connected to outputs of neurons. As an important and flexible Node; y (enantiomeric excess), can be presented as $y_{j,i}$, which means it is the ith input of neuron j. It could be also employed as y_j to describe the output of neuron j. The output node of neuron j is calculated as given in Eq. (9).

$$y_j = f_j\left(net_j\right) \tag{9}$$

where f_j is the activation function of neuron j and net value net_j is the sum of weighted input nodes of neuron j which can be presented by Eq.(10).

$$net_j = \sum_{i=1}^{ni} w_{j,i}\, y_{j,i} + w_{j,o} \tag{10}$$

where, $y_{j,i}$ is the ith input node of neuron j, weighted by $w_{j,i}$, and $w_{j,o}$.

RESULTS AND DISCUSSION

In organic chemistry, the bioreduction of acetophenone to the corresponding (*R*)- or (*S*)-1-phenylethanol as acetophenone is one of the main bioreduction reactions. Its substituted derivatives could be employed as significant resources for the synthesis of many pharmaceutical substances [26-28]. In a similar way, use of whole microbial cells in bioreduction reaction is considered as one of the critical methods. [26] Up to now, the potentials of some strains to bioreduce have been examined and they were proved to be influential entire cell biocatalysts. [26, 29,30] However, some cultural parameters for example, pH, temperature, incubation period and agitation speed remarkably influence the success of complete cell implications, which reveals to necessitate for their optimization with respect to get the highest yield of *EE* (%). [26-28, 31, 32] It is also important to choose the most suitable strain in terms of achieving proper bioreduction and safety. Lactic Acid Bacteria (LAB) are of GRAS status, which makes them one of the significant microbial species regarding whole cell implementations. [29]

In the present work, machine learning algorithm was implemented for the optimization of the experimental factors; e.g. pH, temperature, incubation period, and agitation level as input values to maximize the enantiomeric excess (*EE*) values using a previously obtained data set with the whole cell biocatalyst *Lactobacillus senmaizukei* as a sourdough isolate LAB that was employed to bioreduce acetophenone [21]. Table 1 indicates the empirical layout for the factors to be evaluated and enantiomeric excess (*EE*) values acquired in each experimental run determined previously [21]. These runs in the machine learning approach were pH of 4.5–7.5, incubation temperature of 25–40 ºC, incubation period of 24–96 h and agitation level 50–200 (Table 1). In this study, machine learning algorithms were used to optimize for bioreduction of acetophenone asymmetrically employing entire cell of *Lactobacillus senmaizuke* as explained below. In this respect, finding the most suitable solution is of primary importance, in terms of improvement of the solution. As the solution improves, the combination coefficient, δ is decreased, the LM method approaches the Gauss-Newton method, and the solution usually accelerates to the local minimum. [33,34] As it appears in Figure 4, the LM algorithm carries out a combined training process: around the area with complex curvature, the LM algorithm switches to the steepest descent algorithm till the local curvature is suitable to provide a quadratic approximation; then it approximately becomes the Gauss–Newton algorithm, which can accelerate the convergence importantly. Sum square error (SSE) method was employed to analyse the training process. For all training patterns and network outputs, the SSE is computed by Eq. (11).

$$E\left(x,w\right) = \frac{1}{2}\sum_{p=1}^{p}\sum_{m=1}^{M} e_{p,m}^2 \tag{11}$$

Where, x is the input vector of pH (3.5–7.5), temperature (20–40 °C), incubation period (0–96 h) and agitation speed (50–250 rpm), w is the weight vector, $e_{p,m}$ is the training error at output m when applying pattern p and it is defined as in Eq. (12), m is the index of outputs, from 1 to M, where M is the number of outputs.

$$e_{p,m} = d_{p,m} - o_{p,m} \tag{12}$$

where, *d* is the desired output vector for Enantiomeric excess (%), *o* is the actual output vector for Enantiomeric excess (%). Considering the nodes and the links between the output node y_j of a hidden neuron *j* and network output o_m, a complex nonlinear relationship (see Figure 3) exists that can be defined simply o_m □□F_m, $j(y_j)$, where o_m is the *m*th actual output of the network representing the enantiomeric excess (*EE*, %). F_m, $j(y_j)$ is a complex nonlinear function, its complexity depends on the number of other neurons which are amidst neuron *j* and network output *m*.[24,25]

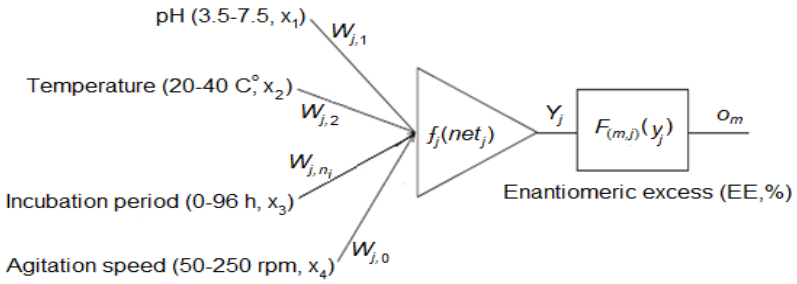

Figure 3. The neurons and nodes representing ANNs of inputs for Enantiomeric excess (%)

The training process was initiated as shown in Figure 2 (a) and the final training was carried out as it appears in Figure 2(b). The training, testing and validation were converged at the 3 Epochs with the validation performance of 92.3206. The outcome is comprehensible due to the succeeding assessments that the final mean-square error and the absolute are small, it seems after training they fall to 0.611236% and 0.080739%, respectively. It is also clear that the test set error and the validation set error have similar characteristics, for instance, no significant over-fitting has occurred by iteration thirteen where the best validation performance has occurred. On the other hand, Figure 4 indicates that the output tracks the targets for training correlation coefficient (*R*), testing *R* and validation *R*; the value of *R* is 1 for training, and 0.91227 for validation of training. Similarly, the value of *R* for testing is 0.97948, and 0.98103 for validation.

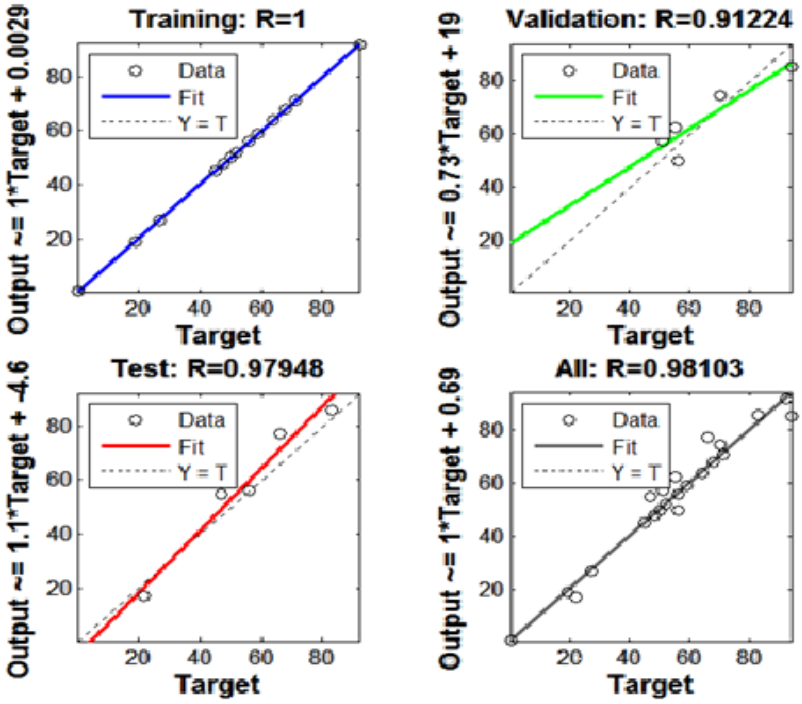

Figure 4. The regression plots for the output in terms of training, validation, and test data

It should be noted here that a too large value of the correlation coefficient (*R*) of determination does not essentially connote that the factor values were calculated with big certainty. The high value of *R* purely designates that the fit has a high degree of correlation with the data. In this study high *R* value is due to comparatively small measuring noise (data values obtained from experiments) and a fit that runs through the data points. It also depicts that these parameters have a high degree of correlation with one another, implicating that a shift in one factor will approximately lead to alterations in the other factors.

A histogram of the divergence amidst the data values and the curve-fit is indicated in Figure 5. It depicts a normally distributed histogram for training, testing and validation data. It is very suitable and appropriate to employ the best available prediction of the targeted factors as the beginning estimation. In default of tangible understanding for a curve-fitting problem, a comprehensible beginning estimation might be obtained by roughly categorizing the factor space and calculating the most suitable combination of factor values.

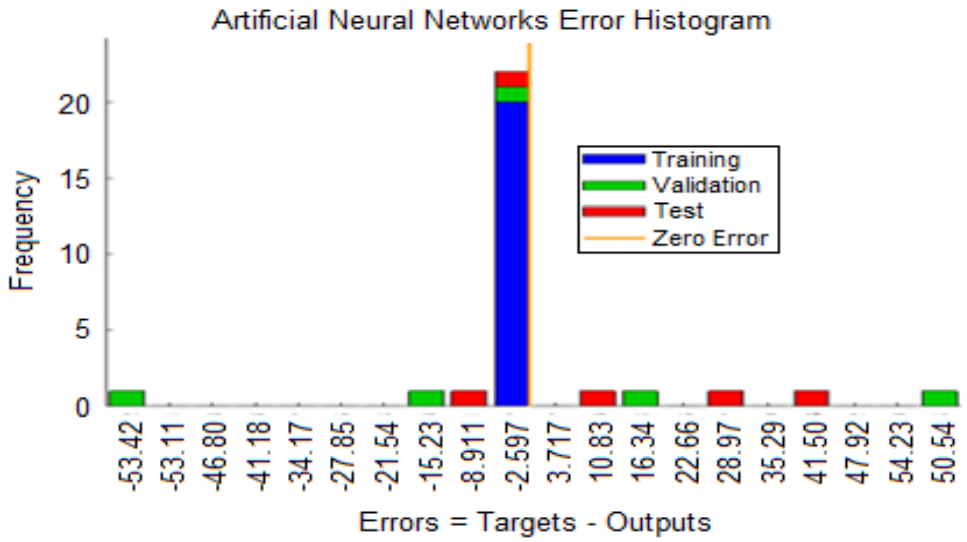

Figure 5. ANNs Error Histogram for Training, Testing and Validation

It is aimed to evaluate unknown samples for enantiomeric excess (*EE*). A neural network approach was produced by employing the same data group utilized to turn out as an ANN training set. The repetitive data was not contained in the training set since ANN does not go well with redundant data values. Matlab was used to generate the ANN model. The input layer contains the absorbance of each pH (3.5–7.5), temperature (20–40 °C), incubation period (0–96 h) and agitation speed (50–250 rpm), and the output parameter was the *EE*. A multilayered perceptron (BPMLP) network with 4 inputs, 8 processing units in the hidden layer, and one output was employed for our analysis. The network was trained by back-propagation algorithms that use the LM algorithm, which minimize the divergence between the input and the output findings. The outcome values predicted by the BPMLP algorithm were converted to *EE* and are recorded in Table 1.

The *EE* error is reasonable because repetitive data noisy data were not used during training testing and validation. 60% of data was used for training, 20% for testing and 20% of data was used for validation. Without including the outlier, the average absolute error was found 0.080739 %, and the sum of the squared errors was found 0.611236 %. The *EE* error can be further reduced by using a larger training data set. Even with this lowest sum of square error, the machine learning algorithm of Artificial Intelligence modeling could be efficiently used to maximize *EE* values. Accordingly, the maximum *EE* (95.5 %) values could be achieved at the levels of 5.7 of pH, 35.5 ºC of temperature, 76 h of incubation period and 240 rpm of agitation speed (Table 2).

Table 2. Experimental factors that maximize enantiomeric excess as calculated by machine learning algorithm

	Experimental factors				Maximization by machine learning algorithm
Response	pH, (x_1)	Temperature (°C), (x_2)	Incubation period (h), (x_3)	Agitation speed (rpm), (x_4)	Maximum
Enantiomeric excess (%)	5.7	35	76	240	95.5

Importantly, the optimized conditions were experimentally tested and validated and similar *EE* (~ 95.5 %) value was obtained, as can be seen in the supporting file HPLC results.

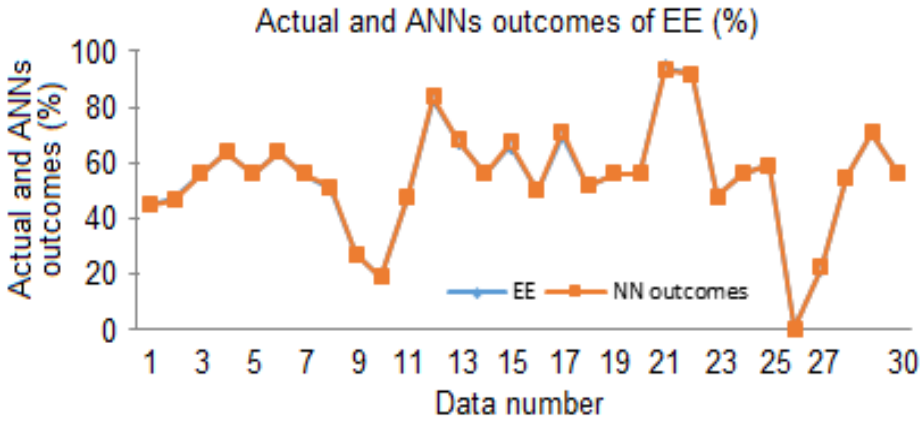

Figure 6. The enantiomeric excess (*EE*) outcomes of actual and ANN

Regarding the success and optimization procedure, the ANN method is very fast once the network could be established by using a training set, testing and validation set for a particular analysis. Both *EE* values of unknown samples could be accurately provided with a family of chiral hosts and indicator combinations with ANN. Figure 6 shows the outcomes of actual enantiomeric excess determined in the laboratory and by the ANN program. The average error was found 0.080739 % which is very minor; hence the ANN can be used for the estimation of the *EE* where certain inputs are difficult and expensive to determine in the laboratories.

CONCLUSION

LM is a well assumed optimization method and works enormously well in practice. [23, 24] It is becoming an effective optimization tool for medium sized nonlinear modelling problems. The findings depicted that the ANN can be used for the estimation of the *EE* where the inputs are difficult and expensive to be determined in the laboratories, in the cases where there are quadratic effects on the experimental factors with respect to pH, temperature, incubation period and agitation speed.

This study demonstrated that the effects of the culture conditions; namely, pH, temperature, incubation period and agitation speed on the *EE* (%) could be well optimized by machine learning. The ANN results in this study demonstrated the significance of each experimental factor. The tested parameters were found to controversially influence the *EE* (%) values based on these factors. For the most optimum bioreduction of the sourdough isolate *Lactobacillus senmaizuke*, the optimized conditions should be adjusted to pH of 5.7, temperature of 35 ºC, incubation period of 76 h and agitation speed of 240 rpm, which would result in maximum *EE* (95.5 %) values. These findings are important as they reveal the importance of the optimisation for the whole-cell applications a biocatalyst in bioreduction.

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