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A combined artificial neural network modeling–particle swarm optimization strategy for improved production of marine bacterial lipopeptide from food waste

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ABSTRACT

In the present study, an artificial neural network (ANN) modeling coupled with particle swarm optimization (PSO) algorithm was used to optimize the process variables for enhanced lipopeptide production by marine *Bacillus megaterium*, using food waste. In the non-linear ANN model, temperature, pH, agitation and aeration were used as input variables and lipopeptide concentration as the output variable. Further, on application of PSO to the ANN model, the optimum values of the process parameters were as follows: pH = 6.7, temperature = 33.3 °C, agitation rate = 458 rpm and aeration rate = 128 L h^{-1}. Significant enhancement of lipopeptide production from waste by about 46% (w/v) with 20 times reduction in operating cost compared to the conventional synthetic medium was achieved under optimum conditions. Thus, the novelty of the work lies in the application of combination of ANN–PSO as optimization strategy to enhance the yield of a fermentative product like lipopeptide biosurfactant from waste.
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1. Introduction

Lipopeptide biosurfactants are synthesized non-ribosomally by the catalytic action of multi-modular enzyme complexes. Surfactin, the most studied lipopeptide biosurfactant is synthesized by a peptide synthetase complex known as surfactin synthetase. This enzyme catalyses the incorporation of seven amino acids into the peptide moiety of surfactin and subsequently acyltransferase enzyme links the peptide into a hydroxy fatty acid to form lipopeptide [1]. Bacteria are the well known producers of lipopeptide biosurfactants, dominated by *Bacillus* species. Lipopeptides of marine bacterial origin have been studied extensively in the last few years [2–4]. These biomolecules are endowed with attributes of powerful surface active agents and are credited with an attractive list of potential applications in the areas of healthcare, energy and environment [5–7]. Despite their versatile properties and myriad applications, high production cost complicated by low product yields impedes their production on a large scale. The cost of raw materials accounts for approximately 30% of total production cost in most biotechnological processes including those of lipopeptide biosurfactants [5]. Therefore, research endeavors have been directed toward utilizing renewable and low cost raw materials for the production of lipopeptides. Various low cost raw materials are reported to facilitate lipopeptide production including cassava wastewater [8], potato process effluents [9] and desizing wastewater [10].

An essential step toward making any bioprocess development endeavor robust and techno-economically sound is to optimize the physicochemical parameters that influence the overall productivity of the process. Media components along with environmental parameters such as pH, temperature, aeration and agitation play a synergistic role in controlling cell growth and lipopeptide production [11,12]. Among various statistical optimization techniques, response surface methodology (RSM) has been extensively employed in the optimization of lipopeptide production [11–15]. However, in some cases, complex non-linear biological interactions cannot be completely described by using second-order polynomial model based on RSM [16,17].

Hence, a more advanced modeling and optimization technique such as artificial neural network modeling coupled with genetic algorithm has been successfully implemented to optimize multivariate non-linear bioprocesses [18,19]. Artificial neural network (ANN) – an emulation of biological neural networks – is used to construct models for predicting outputs for a new input data set. The merits of ANN based models were discussed in earlier reports [18,20]. Since GA suffers from one major shortcoming as

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it destroys previous information between successive generations, a more robust algorithm that can deal with relatively small population size and can help converge at the optimal solutions very quickly while memorizing the previously known good solutions between generations is in great demand. Particle swarm optimization (PSO) is one such population-based evolutionary algorithm that can be more effectively used for solving a non-linear problem involving multiple variables. It is actually inspired by the movement patterns exhibited by living creatures, such as bird flocking and fish schooling wherein they share information among themselves for better living. The particles in PSO follow a similar trend to share information among them and thereby, develop an evolutionary advantage [21]. Constructive cooperation, information sharing, inexpensive computation (requires low memory and CPU speed requirements), and easy implementation are few other attractive properties of PSO [17,22]. Owing to its robustness, PSO is now being applied to solve various non-linear problems with multiple variables in majority of engineering disciplines and has gained the status of a potential competitor of frequently used GA [20]. Since there are no reports on the use of ANN–PSO optimization technique to enhance lipopeptide biosurfactant production, the present work is thus aimed at optimizing the process parameters by ANN modeling coupled with PSO algorithm to maximize lipopeptide concentration using an inexpensive substrate like noodle processed water to improve the overall economy of lipopeptide production.

2. Materials and methods

2.1. Microorganism, medium and growth conditions

Bacillus megaterium, a marine bacterium, isolated from Andaman and Nicobar Islands, India was used in this study. About 10% (v/v) inoculum was developed in 200 mL of Zobell marine broth in 1 L Erlenmeyer flask and incubated in an incubator shaker at 180 rpm and 37 °C for 12 h. Initial screening of different agro-based substrates showed that noodle processed water (NPW) supplemented with chemical fertilizers (CF) exhibited good lipopeptide production and hence, NPW-CF was used as production medium for further studies [23]. NPW was centrifuged at 10,000 rpm for 10 min to separate the solids present in it and the supernatant containing good amount of carbohydrates (~35 g L^{-1}) was used for lipopeptide production. The composition of the production medium used in the current study is as follows (in g L^{-1}): total carbohydrates from NPW – 30, urea – 4.5, di-ammonium phosphate – 1.6, single superphosphate – 0.1, muriate of potash – 0.1, magnesium sulfate (Agriculture grade) – 0.7, ferrous sulfate (Agriculture grade) – 0.18.

2.2. Isolation and analysis of lipopeptide biosurfactant

The culture broth was harvested after fermentation and the cells were separated by centrifuging at 10,000 rpm for 15 min. The supernatant was acidified to pH 2 by adding 6 N HCl and was kept at 4 °C overnight. The precipitate was lyophilized to get crude dry biosurfactant. The crude methanol extract of the biosurfactant and standard surfactin from sigma were spotted manually on a silica gel 60 G-254 plate and were developed in a solvent system containing chloroform, methanol, acetone and acetic acid in ratio 80:10:6:1 (v/v). After development, a densiometric scan was performed at 210 nm to detect biosurfactant as reported earlier [24].

2.3. Optimization of lipopeptide production in batch fermentor

2.3.1. Statistical experimental design

Lipopeptide production was carried out in a 3.7 L fermentor (Model: KLF-2000; Make: BioEngineering, Wald, Switzerland) with a working volume of 2 L. The range and the levels of the four critical process variables, namely, pH, temperature, agitation and aeration are given in Table 1. A central composite design (CCD) was employed for four factors and the experimental design (Table 2) was obtained by using Design Expert version 7.0. The process parameters were varied on the basis of the experimental design and controlled automatically. Sampling was done every 4 h for biomass and lipopeptide analysis.

Table 1
Experimental range and levels of independent variables.

Independent variable	Level				
	−2	−1	0	+1	+2
pH	5	6	7	8	9
Temperature (°C)	25	30	35	40	45
Agitation (rpm)	0	150	300	450	600
Aeration (L h^{-1})	0	50	100	150	200

2.3.2. Artificial neural network modeling

A multilayer perceptron feed-forward neural network with error back propagation (BP) is employed in the present study using MATLAB version 7.14 (Mathworks Inc., Natick, USA). The network essentially consists of an input layer, a hidden layer and an output layer. The inter-connection between the neurons in each layer is defined by weights and biases. ANN learns (updates its weights) the cause effect relationship between input and output variables from the given set of data and tries to minimize the error between the target data (given output) and simulated output. The BP algorithm prevents the recurrence of the error by iteratively feeding the error backwards through the network. The network was trained using mean squared error (MSE) as performance index (Eq. (1)). During training, ANN executes an activation function (Eq. (2)) that sums up the product of the weight and input of each neuron

Table 2
Central composite design of four process variables and corresponding experimental values.

Run order	pH	Temp (°C)	Agitation (rpm)	Aeration (L h^{-1})	Crude lipopeptide (LP) concentration (g L^{-1})
24	6	30	150	50	2.3
15	8	30	150	50	1.8
27	6	40	150	50	1.9
6	8	40	150	50	1.7
30	6	30	450	50	3.4
17	8	30	450	50	2.9
13	6	40	450	50	3
4	8	40	450	50	2.6
29	6	30	150	150	3.6
16	8	30	150	150	2.8
10	6	40	150	150	2.7
14	8	40	150	150	1.9
19	6	30	450	150	5
20	8	30	450	150	3.7
2	6	40	450	150	4.3
1	8	40	450	150	3.9
11	5	35	300	100	3.1
3	9	35	300	100	0.8
18	7	25	300	100	3.5
25	7	45	300	100	2.5
9	7	35	0	100	0.7
21	7	35	600	100	3.3
28	7	35	300	0	0.4
12	7	35	300	200	4.7
7	7	35	300	100	4.5
23	7	35	300	100	4.4
8	7	35	300	100	4.5
5	7	35	300	100	4.5
26	7	35	300	100	4.4
22	7	35	300	100	4.5

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