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# User-friendly optimization approach of fed-batch fermentation conditions for the production of iturin A using artificial neural networks and support vector machine

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ABSTRACT

*Background:* In the field of microbial fermentation technology, how to optimize the fermentation conditions is of great crucial for practical applications. Here, we use artificial neural networks (ANNs) and support vector machine (SVM) to offer a series of effective optimization methods for the production of iturin A. The concentration levels of asparagine (Asn), glutamic acid (Glu) and proline (Pro) (mg/L) were set as independent variables, while the iturin A titer (U/mL) was set as dependent variable. General regression neural network (GRNN), multilayer feed-forward neural networks (MLFNs) and the SVM were developed. Comparisons were made among different ANNs and the SVM.

*Results:* The GRNN has the lowest RMS error (457.88) and the shortest training time (1 s), with a steady fluctuation during repeated experiments, whereas the MLFNs have comparatively higher RMS errors and longer training times, which have a significant fluctuation with the change of nodes. In terms of the SVM, it also has a relatively low RMS error (466.13), with a short training time (1 s).

*Conclusion:* According to the modeling results, the GRNN is considered as the most suitable ANN model for the design of the fed-batch fermentation conditions for the production of iturin A because of its high robustness and precision, and the SVM is also considered as a very suitable alternative model. Under the tolerance of 30%, the prediction accuracies of the GRNN and SVM are both 100% respectively in repeated experiments.



## 1. Introduction

Produced by *Bacillus subtilis*, the nonribosomal lipopeptide antifungal antibiotic iturin A is structurally composed of two parts. The first part consists of seven amino acid residues (L-Asn–D-Tyr–D-Asn–L-Gln–L-Pro–D–Asn–L-Ser) which are formed into a peptide circle. The second part is a hydrophobic tail with 11–12 carbons [1,2,3]. In terms of treating both human and animal mycoses, iturin A has been showed to be a potential bio-resource due to its wide-scale-spectrum antifungal activity [4,5]. According to recent research, the iturin A can also be applied as a controlling agent to fight against plant pathogens causing a decrease in crop production, such as southern corn leaf blight [6].

During the past decades, researchers have paid much attention to the practical production of iturin A due to its foreseeable potential in biological fields. In order to increase the yield of iturin A, the optimization method is commonly adopted in creating better fermentation conditions. For decades, the optimization of fermentation has been studied in many ways [7,8]. In a laboratory environment, the majority of the methods to optimize the fermentation process are largely based on data obtained from a large amount of experimental works, which cannot be used in practical applications. Additionally, the statistic-based methods such as the orthogonal experiment method and response surface methodology (RSM) [9] cost more manpower and resources than expected. In order to gain statistics that are suitable for practical production, researchers brought up the uniform design (UD) method. So far, the UD method has been successfully applied in many optimization processes [6,10,11]. Compared with the traditional statistical methods, the UD can enormously save manpower and resources in the lab by reducing the number of essential experiments in different dimensions and allows as many different levels of factors as it can [6].

With the development of artificial intelligence (AI), artificial neural networks (ANNs) have been widely applied in predictive modeling. With a comparatively higher accuracy in modeling and better ability in generalization, ANNs are able to simulate the bio-process and predict the results [12,13,14,15]. Compared with

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the traditional statistical methods, ANNs can also model all non-linear multivariate functions, while the traditional statistical methods can only model the quadratic functions [16,17,18]. Also, it is reported that the ANNs are more accurate than the RSMs in many cases [19,20]. Normally, UDs have relatively-representative and regularly-distributed patterns. Based on these patterns with high quality, ANNs are also able to establish equally accurate models with a comparatively smaller amount of data than it is supposed to require obtain.

Despite the advantages of ANNs modeling, few studies have reported using ANNs to reduce the number of experiments. An ANN model was established based on UD data was conducted by Peng and colleagues [6]. In their research, the ANN model based on UD data was adopted in the optimization of iturin A yield and a comparison of the ANN-GA methods and the UD methods was conducted for the first time. Adopted widely during variable chemical process [21,22], this method can be effectively used for applications. However, as a technician, one may find it difficult to use this method to practical applications because of its complexity. People may feel confused using related approaches. Here, an alternative series of user-friendly ANNs and a support vector machine (SVM) are proposed to seek a better optimization method in order to increase the yield of iturin A based on the data from Peng's research [6]. We aim at creating more alternative methods to improve the simplification of the fed-batch fermentation conditions for the production of iturin A, so that the maneuverability of the practical applications can be improved using novel modeling methods.

## 2. Materials and methods

### 2.1. Fed-batch fermentation of iturin A

According to Peng and colleagues' research [6], the separated *B. subtilis* ZK8 strain was used for the production of iturin A. The seed culture-medium contained 2.86 g/L $KH_2PO_4$, 3 g/L $MgSO_4$, 25 g/L glucose and 30 g/L peptone. The slant culture-medium contained 1.5 g/L $K_2HPO_4$, 1.8 g/L agar, 1.8 g/L $MgSO_4 \cdot 7H_2O$, 20 g/L peptone and 10 mL/L glycerol. The fermentation culture-medium was prepared with 0.79 g/L $KH_2PO_4$, 0.8 g/L yeast extract, 2.4 g/L soybean protein powder hydrolysate, 3.8 g/L $MgSO_4$ and 31 g/L glucose. Strain ZK8 was activated in the slant culture-medium. The activated strain was then inoculated and incubated in the seed culture medium in a shaker at 30°C with 150 rpm for 20 h. Then, the seed culture was inoculated in fermentation culture by 10% amount of inoculum for 48 h at 30°C with 150 rpm. After 24 h of fermentation, the asparagine (Asn), glutamic acid (Glu) and proline (Pro) were added to the broth in different concentrations [6]. The yield of iturin A was determined by titer measurement and the cylinder-plate method was used to measure the titer of iturin A [6,23,24,25]. According to the experimental results [6], statistical results were obtained (Table 1).

### 2.2. ANNs

ANNs [26,27,28] are powerful machine learning techniques with the functions of estimation and approximation based on the inputs. Interconnected artificial neural networks usually consist of neurons that can calculate values from inputs and adapt to different situations. Therefore, ANNs are capable of numeric prediction and pattern recognizing. Recent years, ANNs have gained wide popularity in inferring a function from observation especially when the data or the task is too complicated to be dealt with human brains. In our studies, multilayer feed-forward neural networks (MLFNs) and general regression neural network (GRNN) were used for developing alternative models for optimizing the fed-batch fermentation conditions of iturin A.

**Table 1**
Statistical experimental results of the amino acid concentration (mg/L) and iturin A titer (U/mL) (data extracted from Peng's research [6]).

| Statistical item | Factor (mg/L) | | | Iturin A titer (U/mL) |
|---|---|---|---|---|
| | Asn | Glu | Pro | |
| MIN | 50 | 200 | 50 | 10,108 |
| MAX | 200 | 400 | 200 | 13,064.1 |
| AVERAGE | 119.6 | 293.52 | 119.6 | 12,033.2 |

#### 2.2.1. MLFNs

MLFNs trained with a back-propagation learning algorithm, are the most popular neural networks [29,30,31]. They are applied to a wide variety of chemistry related problems [29].

An MLFN model consists of neurons that are ordered into layers (Fig. 1). The first layer is called the input layer, the last layer is called the output layer, and the layers between are hidden layers. For the formal description of the neurons we can use the so-called mapping function $\Gamma$, that assigns for each neuron $i$ a subset $\Gamma(i) \subseteq V$ which consists of all ancestors of the given neuron. A subset $\Gamma(i)^{-1} \subseteq V$ consists of all predecessors of the given neuron $i$. Each neuron in a particular layer is connected with all neurons in the next layer. The connection between the $i$th and $j$th neuron is characterized by the weight coefficient $\omega_{ij}$, and the $i$th neuron by the threshold coefficient $\vartheta_i$ (Fig. 2). The weight coefficient reflects the importance degree of the given connection in the neural network. The output value of the $i$th neuron $x_i$ is determined by [Equation 1 and Equation 2]. It holds that:

$$x_i = f(\xi_i) \qquad \text{[Equation 1]}$$

$$\xi_i = \vartheta_i + \sum_{j \in r_i^{-1}} \omega_{ij} x_j \qquad \text{[Equation 2]}$$

where $\zeta_i$ is the potential of the $i$th neuron, and function $f(\zeta_i)$ is the so-called transfer function (the summation in [Equation 2] is carried out over all neurons $j$ transferring the signal to the $i$th neuron). The threshold coefficient can be understood as a weight coefficient of the connection with formally added neuron $j$, where $x_j = 1$ (so-called bias).

For the transfer function, it holds that

$$f(\zeta) = \frac{1}{1 + \exp(-\zeta)}. \qquad \text{[Equation 3]}$$

The supervised adaptation process varies the threshold coefficient $\vartheta_i$ and weight coefficient $\omega_{ij}$ to minimize the sum of the squared

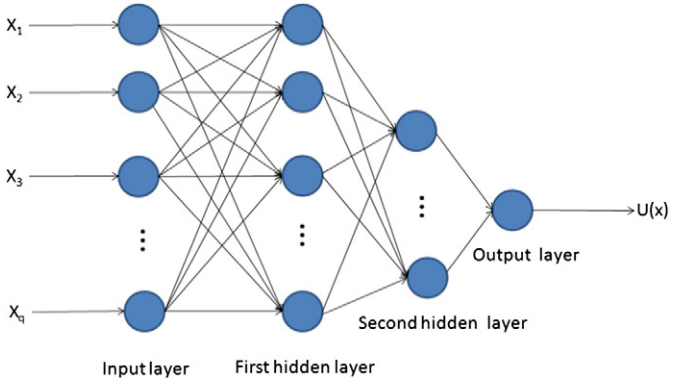


**Fig. 1.** Structure of the MLFN.