Bioresource Technology 138 (2013) 345–352

Contents lists available at SciVerse ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Time-dependent fermentation control strategies for enhancing synthesis of marine bacteriocin 1701 using artificial neural network and genetic algorithm

Jiansheng Peng [1], Fanmei Meng [1], Yuncan Ai *

State Key Laboratory of Biocontrol, School of Life Sciences, Sun Yat-sen University, Guangzhou 510275, PR China

HIGHLIGHTS

- Modeled fermentation process using artificial neural network on five batches of 5L-stirred-tank.
- Optimized the fermentation parameters using a time-dependent strategy.
- Obtained an optimal regulation trajectory using the genetic algorithm.
- Performed 25 batches more fermentation under the optimal trajectory guideline.
- Confirmed the dissolved oxygen level most significantly impacting on modeling.

ARTICLE INFO

Article history:
Received 13 January 2013
Received in revised form 26 March 2013
Accepted 29 March 2013
Available online 6 April 2013

Keywords:
Bioprocess optimization
Artificial neural networks
Genetic algorithms
Fermentation

ABSTRACT

The artificial neural network (ANN) and genetic algorithm (GA) were combined to optimize the fermentation process for enhancing production of marine bacteriocin 1701 in a 5-L-stirred-tank. *Fermentation time*, *pH value*, *dissolved oxygen level*, *temperature* and *turbidity* were used to construct a "5–10–1" ANN topology to identify the nonlinear relationship between fermentation parameters and the antibiotic effects (shown as in inhibition diameters) of bacteriocin 1701. The predicted values by the trained ANN model were coincided with the observed ones (the coefficient of R^2 was greater than 0.95). As the *fermentation time* was brought in as one of the ANN input nodes, fermentation parameters could be optimized by stages through GA, and an optimal fermentation process control trajectory was created. The production of marine bacteriocin 1701 was significantly improved by 26% under the guidance of fermentation control trajectory that was optimized by using of combined ANN–GA method.

1. Introduction

Marine bacteriocin 1701, a metabolite of the marine *Brevibacterium casei* strain, has strong antibacterial activity against a multiple drug-resistant strain EU(3)25 (resistant to 100 μg/mL ampicillin, 15 μg/mL tetracycline, 25 μg/mL chloramphenicol, 50 μg/mL streptomycin and 60 μg/mL erythromycin). Multiple drug-resistant bacteria were emerging in coastal waters (Gulkowska et al., 2007; Li et al., 2012; Vignesh et al., 2012) due to the discharge of large amounts of domestic sewage (Shahidul Islam and Tanaka, 2004; Vignesh et al., 2012; Williams, 1996) and the overuse of antibiotics in coastal aquaculture (Cabello, 2006; Di Cesare et al., 2012). These multiple drug-resistant bacteria can seriously infect aquaculture farmers (Buschmann et al., 2012; Defoirdt et al., 2007) and upset the ecological balance of coastal waters (Sarmah et al., 2006; Zheng et al., 2012). Marine bacteriocin 1701 with strong antibacterial activity is plausible for preventing and curing bacterial infections.

However, synthesis levels of marine metabolites are usually quite low (Davidson, 1995), thus the optimization of fermentation by way of a stirred-tank is essential to enhance production in order to purify compounds and characterize structures. Many factors controlling fermentation process have complex effects on the production (Lübbert and Bay Jørgensen, 2001), and statistical methods such as response surface method (RSM) are widely used (Cruz et al., 2012; Mao et al., 2011) to characterize such interactive effects. The development of accurate models for a microbial fermentation process is still a critical challenge (Singh et al., 2008), mainly due to the non-linear nature of biochemical network interactions. Machine learning techniques including artificial neural network (ANN) and genetic algorithm (GA) can mimic different aspects of biological information processing and have been proved effective in data modeling for the optimization of fermentation processes (Franco-Lara et al., 2006; Silva et al., 2012). ANN is a typical mathematical model simulating the structure and function

* Corresponding author. Tel.: +86 020 84110929; fax: +86 020 34027366.
E-mail address: lssayc@mail.sysu.edu.cn (Y. Ai).
[1] These authors contributed equally to this work.

0960-8524/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.biortech.2013.03.194

of human neural networks (Patnaik, 1999) which is powerful in dealing with a non-linear modeling and has been widely used in biological technology (Patnaik, 1999). GA is a stochastic global search algorithm based on the principles of natural biological evolution (Goldberg and Holland, 1988) and has been widely used for more complex optimizations in bioprocess engineering (Ronen et al., 2002; Sarma et al., 2009). The combined ANN–GA method has been proved superior to RSM (Desai et al., 2008; Nelofer et al., 2012) and effective in modeling and optimization with various bioprocess systems (Chen et al., 2004; Sathish and Prakasham, 2010). For example, the ANN–GA based approach was used for the optimization of medium in shaking-flask fermentation (Zafar et al., 2012a), and further applied to the optimization of agitation and aeration rates in a 3-L-stirred-tank (Zafar et al., 2012b) fermentation for the production of P(3HB-co3HV) by *Azohydromonas lata* MTCC 2311. The RSM based data were further optimized by using the ANN-GA method to improve the productions of marine biosurfactant by approximately 70% (Sivapathasekaran et al., 2010), alkaline protease by 2.5 times (Rao et al., 2008), and glucansucrase by 6.0% (Singh et al., 2008). Similarly, the combined ANN–GA method was used to optimize the mixed substrates for biogas production resulting in an increase of 8.64%, and an early biogas production initiated on the third day of fermentation, compared to the eighth day in the non-optimized system (Gueguim Kana et al., 2012).

Unlike those methods mentioned above, however, in the present study the fermentation time will be brought in as one of the ANN input nodes so that all parameters regulating an auto-controlled 5-L-stirred-tank could be optimized in a coordinated way focusing on controlling the fermentation stages. According to this assumption, an optimal piecewise control trajectory can be worked out by using the combined ANN–GA method; and the production can be improved under the guidance of such an optimized piecewise control trajectory.

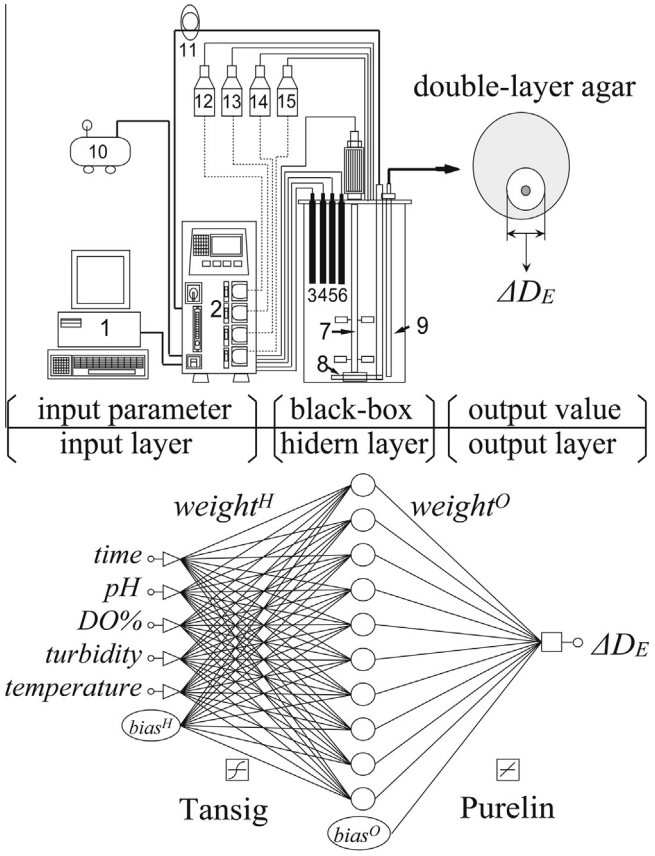

Fig. 1. Schematic diagram of a 5-L-stirred-tank and artificial neural network modeling. 1: Recorder; 2: control panel; 3: turbidity probe; 4: pH electrode; 5: temperature electrode; 6: DO probe; 7: stirrer; 8: air sparger; 9: sampling nozzle; 10: air compressor; 11: air filter; 12: 1 N HCl; 13: 12.5% ammonia; 14: 50% glycerol; 15: antifoamer.

2. Methods

2.1. Microorganism and culture conditions

Marine *B. casei* 1701 strain and multiple drug-resistant strain EU(3)25 were kept in our lab and used in this study. Marine strain 1701 was inoculated into 200 mL of Zobell culture medium in a 500 mL Erlenmeyer flask and incubated in a temperature-controlled incubator shaking at 200 rpm/min, 28 °C, for 24 h (such conditions were optimized previously by using RSM). The batch fermentation was carried out in a 5-L-stirred-tank (Biostat B, Germany) with 3.5 L working volume of production medium and 10% (v/v) inoculums. The optimized production medium for bacteriocin 1701 was composed of (g/L): fish peptone 19, glycerol 8, NaCl 43, NH_4Cl 10.5, and $MgSO_4$ 3.8. The pH values of the culture medium were regulated with 1 N HCl and 12.5% ammonia.

2.2. Assay of antibacterial activity

Samples (2 mL) from the fermented broth were taken at 4-h intervals and centrifuged at 8000 rpm/min for 15 min at 4 °C, and the cell free supernatant was used for estimating the antibacterial activity of the multiple drug-resistant strain EU(3)25, using a double-layer agar technique (Shilo, 1970). The diameter of inhibition zone was used to indicate the synthesis level of marine bacteriocin 1701. The *fermentation time*, *pH value*, *dissolved oxygen level*, *turbidity* and *temperature* were recorded online by the electrodes and probes of 5-L-stirred-tank (Fig. 1).

2.3. Modeling and optimization

2.3.1. ANN modeling

The 5-L-stirred-tank was treated as a black box (Fig. 1) according to black-box theory (Bunge, 1963), ignoring microbial physiological and biochemical process of fermentation, and only focusing on the relationship between input and output values of the stirred tank. The ANN modeling was experimentally proved effective for establishing a complex, non-linear relationship between input and output. Fermentation parameters (*fermentation time*, *pH value*, *dissolved oxygen level*, *temperature* and *turbidity*) were used to construct a "5–10–1" ANN topology to identify the nonlinear relationship between fermentation parameters (input vector) and bacterial inhibition diameter ΔD_E (output vector) (Fig. 1). The connection between the input layer and the hidden layer was achieved by transfer function 'tan–sigmoid' (f_1: tansig), weights ($weight^H$) and bias ($bias^H$) between the input layer and the hidden layer; the connection between the hidden layer and the output layer was achieved by transfer function 'pure–linear' (f_2: purelin), weights ($weight^O$) and bias ($bias^O$) between the hidden layer and the output layer (Fig. 1). The predicted output values (ΔD_E^P) can be described as Eq. (1). The back–propagation algorithm was used to train the ANN (Rummelhart et al., 1986). In this training algorithm, the error between the predicted (ΔD_E^P) and the observed values (ΔD_E^O) was calculated and propagated backward through the network to the weights of each layer. The algorithm regulated the weights continuously in the direction of reducing errors between ΔD_E^P and ΔD_E^O until the errors met the requirement. The ANN stopped training when the mean square error (*MSE*, Eq. (2)) between ΔD_E^P and ΔD_E^O met the pre-set value of 0.005. A trained ANN must be evaluated by testing samples (a dataset not used for