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ORIGINAL ARTICLE

The optimization of *Marasmius androsaceus* submerged fermentation conditions in five-liter fermentor

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Marasmius androsaceus; Submerged fermentation; Plackett–Burman design; Neural network combining genetic algorithm; Desirability function

Abstract Using desirability function, four indexes including mycelium dry weight, intracellular polysaccharide, adenosine and mannitol yield were uniformed into one expected value (*Da*) which further served as the assessment criteria. In our present study, Plackett–Burman design was applied to evaluate the effects of eight variables including initial pH, rotating speed, culture temperature, inoculum size, ventilation volume, culture time, inoculum age and loading volume on *Da* value during *Marasmius androsaceus* submerged fermentation via a five-liter fermentor. Culture time, initial pH and rotating speed were found to influence *Da* value significantly and were further optimized by Box–Behnken design. Results obtained from Box–Behnken design were analyzed by both response surface regression (Design-Expert.V8.0.6.1 software) and artificial neural network combining the genetic algorithm method (Matlab2012a software). After comparison, the optimum *M. androsaceus* submerged fermentation conditions via a five-liter fermentor were obtained as follows: initial pH of 6.14, rotating speed of 289.3 rpm, culture time of 6.285 days, culture temperature of 26 °C, inoculum size of 5%, ventilation volume of 200 L/h, inoculum age of 4 days, and loading volume

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of 3.5 L/5 L. The predicted *Da* value of the optimum model was 0.4884 and the average experimental *Da* value was 0.4760. The model possesses well fitness and predictive ability.

1. Introduction

As an efficient way to produce bioactive metabolites for fungus, submerged fermentation has been studied for years (Saha et al., 2014; Zhou et al., 2014). *Marasmius androsaceus*, a traditional Chinese medicine, possesses analgesic and antioxidant effects. In China, "An-Luo Tong", produced by fermentation mycelium of *M. androsaceus,* has been used as a painkiller for years. However, its large-scale application both as a medicine and asfood is limited by immature artificial cultivation technology. The optimum fermentation conditions of *M. androsaceus* in a fermentor has not been reported yet (Surhio et al., 2014; Naureen et al., 2014).In our previous study, by using chemometric methods, the optimum submerged fermentation medium of *M. androsaceus* was obtained. Moreover, in our group, desirability function combining Plackett–Burman design and Box–Behnken design was successfully applied to optimize submerged fermentation medium of *Paecilomyces tenuipes* N45 and *Saccharomyces cerevisiae* (Dong et al., 2012; Du et al., 2012). Similar to previous studies, desirability function is employed to unite several response data into one expected value which served as the assessment criteria (Dong et al., 2012; Du et al., 2012; Heidari et al., 2014). Generally, following with Plackett–Burman design which is used to identify the important process variables (Giordano et al., 2011), Box–Behnken design is further applied to optimize selected variables and quantify the functional relationship between measured response values and explanatory factors (Dalvand et al., 2014; Safi et al., 2015). As a statistical and mathematical techniques response surface methodology (RSM) is widely applied in the optimization of various processes in food chemistry, chemical engineering, material science and biotechnology (Bezerra et al., 2008; Kiyani et al., 2014; Khaskheli et al., 2015). Moreover, artificial neural network combined with genetic algorithm (ANN-GA) is considered as an alternative modeling technique used in the field of microbiology (Tripathi et al., 2012; Batool et al., 2015). Artificial neural network (ANN) imitates the behavior of neurons in the human brain and possesses advantages including non-linearity, flexible, speed, simplicity, and high accuracy (Manning et al., 2014). Based on the concept of natural selection and genetics, genetic algorithm (GA) is an effective tool to solve complex optimization problems in various scientific and technological fields (Wang and Li, 2014).

Our present study was conducted in an attempt to search an optimal submerged fermentation condition of *M. androsaceus* in a five-liter fermentor by using statistical and mathematical techniques including Plackett–Burman design and Box–Behnken design. Both RSM and ANN-GA were performed to analyze results from Box–Behnken design.

2. Materials and methods

2.1. Strains, agents and equipment

M. androsaceus (CCTCC M2013175) was deposited in China Center for Type Culture Collection, China.

M. androsaceus was cultured in a five-liter full-automatic fermentor (Baoxing Bioscience company, Shanghai, China) using a defined liquid medium containing: 20 g/L sucrose, 10 g/L peptone, 10 g/L yeast extract powder, 1 g/L $MgSO_4{\cdot}7H_2O$, 1 g/L $KH_2PO_4{\cdot}3H_2O$, and 0.1 g/L Vitamin B_1.

All the chemical reagents used in the present experiment for submerged fermentation and effective constituent determination were obtained from Sigma–Aldrich, USA.

2.2. The concentration of effective constituents determination

2.2.1. Measurement of polysaccharide

The amount of total saccharides was determined by anthrone–sulfuric acid method (Leyva et al., 2008). Monosaccharide concentration was measured by 3, 5-2 nitrate salicylic acid (DNS) colorimetry (Teixeira et al., 2012).

The concentration of polysaccharide = The concentration of total saccharides – the concentration of monosaccharide.

2.2.2. Measurement of adenosine

As it is recommended in the Chinese Pharmacopoeia that the adenosine concentration in *M. androsaceus* is detected via high performance liquid chromatography (HPLC) (Chinese Pharmacopoeia Commission, 2010).

2.2.3. Measurement of mannitol

The concentration of mannitol in *M. androsaceus* mycelium was measured using the colorimetric method as described previously (Dong and Yao, 2008).

2.3. The optimization of submerged fermentation conditions of *M. androsaceus* in a five-liter fermentor

2.3.1. Desirability function establishment

Based on desirability function (Kleijnen and Sargent, 2000), the mycelium yield, the concentration of intracellular adenosine, mannitol and polysaccharide were uniformed into one index—*Da* (ranged: 0–1) according to the following equations.

$$d_i = \left|\frac{\hat{y}_i - y_{\min}}{y_{\max} - y_{\min}}\right| \quad \hat{y} > y_{\max},\ \ d_i = 1;\ \ \hat{y} < y_{\min},\ \ d_i = 0 \tag{1}$$

where, $\hat{y}_i$ is the response value of an i analyzed factor.

$$Da = d_1^{w_1} \times d_2^{w_2} \times d_3^{w_3} \times \cdots \times d_i^{w_i} \ldots w_1 + w_2 + w_3 + \cdots w_i = 1 \tag{2}$$

where, w_i is weighted value of index i.