Biocatalysis and Agricultural Biotechnology 10 (2017) 9–12

Contents lists available at ScienceDirect

Biocatalysis and Agricultural Biotechnology

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

Effects of agitation and volume of inoculum on ferulic acid production by co-culture

Kamaliah Abdul Samad*, Norazwina Zainol

Faculty of Chemical and Natural Resources Engineering, Universiti Malaysia Pahang, Lebuhraya Tun Razak, 26300 Gambang, Kuantan, Pahang, Malaysia

ARTICLE INFO

Keywords:
Response surface methodology
Ferulic acid
Banana stem waste
Co-culture
Fermentation

ABSTRACT

Response surface methodology (RSM) has been known as the best tool in evaluating the effects of several variables and their interactions of biochemical and biotechnological processes. The main focus of this research was to study the effects of agitation and volume of inoculum in influencing the ferulic acid production from banana stem waste by co-culture. The optimum conditions for the production process were also determined. RSM with five replicates at the center point was performed to evaluate the contribution of the factors. The *p*-values of 0.0249 and 0.0268 for agitation and volume of inoculum, respectively, indicate the importance of both factors in ferulic acid yield. Agitation at 150 rpm and volume of inoculum of 5% were found optimum in increasing the ferulic acid yield up to 510.24 mg/kg within 24 h. The results demonstrate that the use of RSM is very helpful to study the effects of interactions among the parameters for efficient production of biological products.

1. Introduction

Ferulic acid (FA) is one of the three p-hydroxycinnamic acids present in large quantities in lignocellulosic biomass (Barbara et al., 2015). FA may form the linkage of ester and ether bonds between arabinoxylans and lignins, which are important for plant stability (Bauer et al., 2012).

Several methods have been used to release FA from plant cell wall. Enzymatic hydrolysis is one of the biological methods used for the purpose by hydrolyzing the FA linkages. Faulds and Williamson (1995) reported that a maximum of 95% of total FA was proficiently released from wheat bran after a purified ferulic acid esterase (FAE) from *Aspergillus niger* and a *Trichoderma viride* xylanase were incubated together for five hours. In other research by Uraji et al. (2013), enhanced enzymatic production of FA was achieved by combining several enzymes produced by *Streptomyces*. The reports implied that co-culture may result in increased yield and improved control of product qualities compared to single culture. Huang et al. (2011) indicated that the degradation of plant cell wall by microbial could be increased through synergistic acts between FAE with other hemicellulase such as xylanase.

Thus, the use of co-cultivation together with banana stem waste (BSW) as substrate promises a significant and continuous production of FA because BSW is abundant and can be exploited as an alternative source for this phenolic acid production (Cruz et al., 2001).

Response surface methodology (RSM) has been used to evaluate the effects of several parameters and their interactions as originally described by Box and Wilson (1951). Several studies had been performed to extract FA from several raw materials such in brewer's spent grain (BSG), wheat straw, *Angelica sinensis*, *Cinicifuga racemose,* and paddy straw employing RSM to obtain the optimum yield (Salleh et al., 2011). Ismail and Zainol (2014) successfully determined the optimum condition in extracting FA from BSW using sugar cane press machine. However, to date, the optimization of FA production from BSW by fermentation of co-culture has never been reported. Therefore, it is interesting to improve the FA production by applying optimization tools like RSM. Hence, this research was conducted to study the effect of factors contributed in the release of FA and to optimize the condition process for maximum FA production monitoring.

2. Materials and methods

2.1. Microorganism

Bacillus cereus strain CCM 2010 and *Bacillus thuringiensis* Bt407 from soil were identified by 16S rRNA sequence analysis identification method. The bacterial strains were acclimatized for a month in BSW before isolation. All the strains were incubated at 37 °C for 24 h on nutrient agar plate for inoculum preparation.

* Corresponding author.
E-mail addresses: kamaliahabdulsamad@ymail.com (K.A. Samad), azwina@ump.edu.my (N. Zainol).

http://dx.doi.org/10.1016/j.bcab.2017.01.010
Received 23 August 2016; Received in revised form 23 December 2016; Accepted 30 January 2017
Available online 31 January 2017
1878-8181/

2.2. Substrate

Banana stem waste (BSW) was obtained from banana plantation in Kuantan, Pahang, Malaysia. The stem was cleaned to remove any dirt attached before being used as substrate. The stem was cut into a cube (1 cm). One part of the cut stem and two parts of distilled water were blended. The initial pH of the mixture was adjusted to 9.5 by an addition of 1 M of NaOH. A 250-ml Erlenmeyer flask was used for substrate preparation and the substrate was sterilized by autoclaving at 121 °C for 15 min.

2.3. Preparation of co-culture

Before starting the experiment, bacterial strains of *Bacillus cereus* strain CCM 2010 and *Bacillus thuringiensis* Bt407 were resuscitated by plating onto nutrient agar plate. The strains were incubated at 37 °C for 24 h. A single colony of each strain was inoculated in 10 ml of nutrient broth and incubated for 22 h at 37 °C. The cultures were aseptically transferred into 50 ml of sterile nutrient and incubated for another 22 h at 37 °C. Co-culture of *B. cereus* and *B. thuringiensis* were prepared by adding 25 ml of stationary phase inoculum of single culture each into 1000-ml Erlenmeyer flask containing 500 ml of nutrient broth. The inoculum was incubated at 37 °C for another 22 h in an incubator shaker.

2.4. Experimental set-up

An inoculum of co-culture at stationary phase was inoculated into 250-ml Erlenmeyer flask containing the substrate. Incubation was performed in an incubator shaker at 26 °C with different agitation for 24 h. Samples were collected and centrifuged. Quantification of FA content was done by analyzing the supernatant using high performance liquid chromatography (HPLC). The outputs of the experimental design were analyzed with Design Expert software.

2.5. Response surface methodology

The effect of each factor involved in FA production was studied through RSM by determining the optimum condition. RSM was performed through central composite design (CCD) using Design Expert Software, version 8.0.6 (State-Ease, Inc.). Optimization was performed using two independent variables which were identified as having significant effect on FA production. The independent variables considered were agitation (130–170 rpm) and volume of inoculum (3–7%, v/v) as presented in Table 1.

The experimental data were fitted by regression to a quadratic model as mentioned in Eq. (1):

$$Y=\beta_0 + \sum_{i=1}^{n} \beta_i X_i + \sum_{i=1}^{n} \beta_{ii} X_i^2 + \sum_{i=1}^{n} \cdot \sum_{j=1}^{n} \beta_{ij} X_i X_j + \varepsilon \quad (1)$$

where Y represents the value of the predicted response, β_0 is a constant, β_i, β_j and β_{ij} are the linear, quadratic and interaction coefficients, respectively, and X_i and X_j are the experimental variables which levels are being optimized.

Table 1
Experimental range and levels of the variables in the central composite design.

Variables	Code	Levels of variables				
		−α	−1	0	+1	+α
Agitation (rpm)	A	130	140	150	160	170
Volume of inoculum (%, v/v)	B	3	4	5	6	7

2.6. Model validation

The optimum condition for maximum yield obtained from CCD was validated by confirmation runs performed in 250-ml Erlenmeyer flask. Condition of the experiment was prepared according to the highest FA production. The validity of the model was checked by comparing the experimental and predicted values. The experiment was performed in triplicate.

2.7. Analytical method

Samples were analyzed using HPLC (Agilent 1100 system) equipped with Agilent Zorbaq SB-AQ C18 analytical column. The method of Chamkha et al. (2001) with modification was used to quantify the FA content. An isocratic mobile phase used consisted of acetonitrile, distilled water, and acetic acid (30:69.5:0.5, v/v). The flow rate was set at 0.6 ml/min. The samples were injected via 25-ml injection loop and the FA content was measured using a diode array detector (DAD) at 280 nm. Prior to the analysis, samples were prepared by centrifugation at 5800 rpm for 15 min. The supernatant was filtered into a vial using a 0.45 μm nylon syringe filter.

3. Results and discussion

3.1. Analysis of variance (ANOVA)

Table 2 presents the results obtained from the 13 experiments performed through RSM approach. The FA yield ranged from 460.86 to 510.24 mg/kg. High FA production was achieved at the center point level (standard order 9–13).

The second order polynomial model was utilized to express the FA production as a function of independent factors as shown in Eq. (2):

$$FA = -1350.46 + 23.58A + 40.52B - 0.086A^2 - 9.60B^2 + 0.39AB \quad (2)$$

where the FA represents the response of the FA yield, and A and B are the coded values of agitation and volume of inoculum, respectively. The term of A and B are denoted as the main effects, while AB is the interaction involves in the FA production. Quadratic effects are presented through A^2 and B^2 to imply the presence of curvature in the model.

Table 3 presents the analysis of variance (ANOVA) and regression coefficients. The *F*-value of 60.17 indicated that the model is significant for FA production, while the *p*–values for agitation and volume of inoculum are 0.0249 and 0.0268, respectively. Statistically, the *p*-value of less than 0.05 implies the significance of a model (Ismail and Zainol, 2014). The results indicated that the factors were very important in the FA production. It proposes that the model is satisfactory in predicting

Table 2
Experimental layout for optimization of FA production using central composite design, and the responses obtained from the experiments.

Standard	Variables properties		FA
Order	A	B	(mg/kg)
1	−1	−1	493.31
2	+1	−1	478.66
3	−1	+1	489.01
4	+1	+1	489.86
5	-α	0	475.64
6	+α	0	466.47
7	0	-α	460.86
8	0	+α	473.18
9	0	0	507.90
10	0	0	499.87
11	0	0	506.06
12	0	0	510.24
13	0	0	503.25