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OPTIMIZATION OF CHITINASE PRODUCTION FROM *BACILLUS CEREUS* SMG 1.1 USING RESPONSE SURFACE METHODOLOGY

OPTIMASI PRODUKSI KITINASE BACILLUS CEREUS *SMG 1.1 MENGGUNAKAN RESPONSE SURFACE METHODOLOGY*

Adnan Widodo, Indun Dewi Puspita*,* and *Ustadi
Department of Fisheries, Faculty of Agriculture, Universitas Gadjah Mada

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ABSTRAK

Limbah cangkang udang berpotensi memiliki nilai tambah apabila diolah menjadi kitin dan turunannya. Pengolahan limbah dengan pendekatan biologi lebih disukai dan ramah lingkungan dibandingkan kimiawi. Kitinase merupakan enzim yang berperan penting dalam pengolahan limbah cangkang udang. Bacillus cereus SMG 1.1 telah diisolasi dari produk terasi dan menunjukkan kemampuan produksi kitinase yang baik. Penelitian ini bertujuan untuk mengetahui komposisi optimum media produksi kitinase B. cereus SMG 1.1 melalui Response Surface Method (RSM) menggunakan desain faktorial dengan 15 perlakuan. Optimasi media dilakukan dengan menentukan faktor yang berpengaruh terhadap produksi kitinase melalui desain Plackett-Burman dilanjutkan dengan optimasi konsentrasi media melalui desain Box-Behnken. Pengujian dilakukan untuk mengetahui pengaruh variabel bebas terpilih terhadap aktivitas kitinase. Hasil analisis Plackett-Burman menunjukkan bahwa koloidal kitin, fruktosa, dan $MgSO_4.5H_2O$ merupakan komponen medium yang paling berpengaruh terhadap produksi kitinase B. cereus SMG 1.1. Analisis Box-Behnken menghasilkan model linier yang dapat digunakan untuk memprediksi respon. Nilai respon maksimal didapatkan pada konsentrasi 1,5% koloidal kitin, 0,75% fruktosa, dan 0,075% $MgSO_4.5H_2O$ dengan aktivitas kitinase optimal sebesar 0,0016 U/ml.

Kata kunci: *Kitinase; Kitin; Fruktosa; $MgSO_4.5H_2O$; RSM.*

ABSTRACT

Shrimp shell waste are potential to be processed further into value-added products, such as N-acetylglucosamine (GlcNAc). In the production of GlcNAc, biological approach is preferred and environmentally friendly to chemical treatment. Chitinase is an enzyme that plays a vital role in bioprocessing of shrimp shell waste into GlcNAc. Previously, Bacillus cereus SMG 1.1 was isolated from fermented shrimp paste (terasi) and showed the ability to produce chitinase. This study was designed to determine the optimum medium for the production of chitinase from B. cereus SMG 1.1 through the Response Surface Method (RSM) using a factorial design with 15 treatments. The optimization of the media was carried out by determining the factors that influence the production of chitinase through the Plackett-Burman design followed by optimization of the concentration of the media through the Box-Behnken design. The test was designed to assess the effect of the independent variables on chitinase activity. Placket Burman analysis shows that colloidal chitin, fructose, and $MgSO_4.5H_2O$ were the significant components in the medium affecting the production of chitinase by B. cereus SMG 1.1. Box-Behnken analysis developed a linear model capable of predicting the response. The highest response value was achieved at a concentration of

*Corresponding author: indun_dp@ugm.ac.id

0.75% fructose, 1.5% colloidal chitin, and 0.075% $MgSO_4.5H_2O$ resulted in optimum chitinase activity of 0.0016 U/ml.

Keywords: *Chitinase; Chitin; Fructose; $MgSO_4.5H_2O$; RSM*.

INTRODUCTION

Chitin, the second most abundant natural biopolymer after cellulose, is a copolymer component composed of N-acetyl-D-glucosamine (GlcNAc) and D-glucosamine (GlcN) linked by β -(1,4) glycosidic bonds. Chitin is a structural polysaccharide found in the cell walls of fungi, nematodes, insect exoskeletons, and crustaceans. Every year the production of shellfish and crustaceans from the fisheries processing industry contributes to the accumulation of significant quantities of chitin-rich waste that can cause environmental pollution if not appropriately handled (Wang et al., 2002).

Utilizing chitin-rich waste from the fisheries processing sector is crucial for environmental preservation and providing added value from the waste. Chitin-rich waste can be processed into chitin and its derivatives such as chitin-oligomer and GlcNAc, which can be utilized as antibacterial, antifungal, antihypertensive agents, and as food quality enhancers (Saima et al., 2013). The utilization of chitin waste by chemical methods often involves corrosive chemicals such as strong acids, which can cause environmental pollution. As a result, the waste must be treated carefully to ensure its safety when disposed of in the environment. This additional waste treatment may result in increased processing costs. Additionally, it has been reported that the application of strong acids results in reduced product yields. The enzymatic method may be a viable alternative to chemical methods in producing chitin derivatives. The chitinase enzyme (EC 3.2.11.14) can hydrolyze insoluble chitin into oligomers and their monomer components (Younes & Rinaudo, 2015).

Various organisms produce chitinase for a variety of reasons. In bacteria, chitinase plays a role in nutrition and parasitism while in fungi, protozoa, and invertebrates it plays a role in morphogenesis. Chitinase also has a role in the defense mechanisms of plants and vertebrates (Patil et al., 2000). Bacteria synthesize chitinase to break down chitin and metabolize it as a carbon and energy source (Lien et al., 2007). Members of the genus Bacillus are known for their potential as producers of several degradative enzymes, including chitinase (Singh et al., 2016). *Bacillus* sp. CH2, *B. cereus* GS02, and *B. pumilus* RST25 isolated from various types of chitin-rich soil are able to produce chitinase of 0.31 U/ml (Kumar et al., 2017), 1.68 U/ml (Dukariya & Kumar, 2020), and 59.4 U/ml (Gurav et al., 2017), respectively.

B. cereus SMG 1.1, isolated from fermented shrimp paste (Pramana, 2014), has a chitinase activity of 0.0012 U/ml (Siboro, 2017). This activity value is relatively smaller than several other studies, such as *B. cereus* YQ308 with chitinase activity of 1.1 U/ml (Chang et al., 2003), *B. cereus* CH with the activity of 0.0856 U/ml (Mabuchi et al., 2000), and *B. cereus* TKU027 with an activity of 0.026 U/ml (Liang et al., 2014). The production of *B. cereus* SMG 1.1 chitinase needs to be optimized to increase its production. Optimizing media is crucial for increasing yield and productivity while lowering production costs. In addition, the composition of the medium dramatically affects the production of extracellular microbial chitinase, and its interactions play a vital role in the synthesis of the chitinase enzyme (Park et al., 2005). Optimizing parameters with statistical methods can reduce time and cost. Several statistical and non-statistical methods are available for the optimization of medium constituents. Plackett-Burman and response surface method are the most widely used statistical approaches (Montgomery, 2013).

Ghorbel-Bellaaj et al. (2012) conveyed the optimization results of chitinase *B. cereus* SV1 using response surface method, which resulted in chitinase of 0.0828 U/ml. Research by Rishad et al. (2016) showed that the chitinase activity of *B. pumilus* increased 6.9 times from 3.36 U/ml to 23.19 U/ml af-

ter Box-Behnken optimization. Research by Aounallah et al. (2017)stated that the optimization of *B. licheniformis* AT6 with Box-Behnken produced chitinase of 0.5053 U/ml, an increase of 10 times from the initial condition (0.0503 U/ml).

Dhananjaya (2018) reported the optimal pH and temperature parameters for *B. cereus* SMG 1.1 growth were at pH 8 and 30°C. In this study, the composition of the growth medium of *B. cereus* SMG 1.1 was optimized using a statistical approach. A Plackett-Burman design was used to identify significant variables impacting enzyme production. Afterward, the significant variables were optimized using the Box-Behnken design to identify their optimal level.

METHOD

Medium preparation

The medium used in this study was colloidal chitin in agar and broth. Colloidal chitin was prepared in the manner described by Arnold and Solomon (1986), namely by dissolving 20 grams of powdered chitin in 150 ml of HCl (37%), homogenizing without heating, and filtering through glass wool. The solution was then slowly poured into 800 ml of distilled water at 4^0C and incubated for 24 hours at 4^0C. A whitish precipitate composed of colloidal chitin would appear. The precipitate was rinsed with water to neutral and centrifuged at 6,000 rpm for 10 minutes at 4^0C. The supernatant was discarded, and the precipitate was taken as colloidal chitin ready for use.

Colloidal chitin agar was used at the stage of refreshing bacterial isolates. It was prepared using a modified Hsu & Lockwood (1975) method. Modifications were made by removing FeSO4 from the medium. Chitin agar medium was prepared by dissolving KH_2PO_4 (0.03%) (Merck, Germany), K_2HPO_4 (0.07%) (Merck, Germany), $MgSO_4.5H_2O$ (0.05%) (Merck, Germany), $ZnSO_4$ (0.0001%) (Merck, Germany), $MnCl_2$ (0.0001%) (Merck, Germany), colloidal chitin (2%), and bacto agar (2%) (Merck, Germany) into distilled water and sterilized at 121°C for 20 minutes. Colloidal chitin broth was used for the chitinase production stage. Broth was prepared in the same way as the preparation of colloidal chitin agar, but added with ammonium nitrate (Merck, Germany) and fructose (Merck, Germany) without bacto agar. The composition of the colloidal chitin broth for the components of KH_2PO_4, K_2HPO_4, $MgSO_4.5H_2O$, colloidal chitin, ammonium nitrate (NH_4NO_3), and fructose follows the treatment carried out in the optimization stage.

Inoculum Preparation

B. cereus SMG 1.1 was a collection of the Laboratory of Fishery Products Quality and Safety isolated from fermented shrimp paste (Pramana, 2014). One loop of the isolate was taken from the culture stock and inoculated into 7 ml of Nutrient Broth (NB) (Merck, Germany) aseptically, then incubated at 37°C for 24 hours. After being grown in NB, the isolate was streaked onto a colloidal chitin agar medium and incubated at 37°C until a clear zone appeared on the medium, indicating that the colonies that grew were pure isolates. Single colonies of colloidal chitin agar were taken and re-grown on 7 ml NB medium and incubated at 37°C for another 24 hours. The culture from 7 ml NB was then taken as much as 0.6 ml, transferred to 30 ml NB medium, and incubated in a water bath shaker at 37°C for 6 hours to reach the logarithmic phase (Dhananjaya, 2018).

Measurement of Chitinase Activity

Chitinase activity was tested by taking 2x1 ml of fermentation medium, then centrifuged at 10,000 rpm for 1 minute at 4°C. The two cell-free supernatants were referred to as the sample and negative control. The negative control was boiled treatment at 100°C for 3 minutes to inactivate the enzyme. Furthermore, 0.5 ml of sample and 0.5 ml of negative control were reacted with 1 ml of 1.3% colloidal chitin (in 50 mM phosphate buffer pH 7.4) by being incubated for 30 minutes in a water bath shaker at 37°C, with an agitation speed of 100 rpm. The mixture was heated in boiling water for 3 minutes to stop the reac-

tion, then cooled and centrifuged at 10,000 rpm for 5 minutes (Wang et al., 2012).

The supernatant from the reaction was then tested for its chitinase activity based on the concentration of GlcNAc using the method of Reissig et al. (1955) by taking 250 µl of the supernatant and reacting it with 50 µl of potassium tetraborate (Wako, Japan) pH 9.1 to bind GlcNAc, then boiled for 3 minutes to accelerate the GlcNAc binding reaction. After boiling, the sample was cooled, and 1.25 ml of p-dimethylaminobezaldehyde (DMAB) (Merck, Germany) reagent was added and immediately incubated in a water bath shaker for 30 minutes at 37°C to measure the absorbance using a UV-Vis spectrophotometer (Thermo Scientific, US) at a wavelength of 585 nm. The same treatment was also carried out on the standard GlcNAc (TCI, Japan) solution at various concentrations (0-50 g/ml) to obtain a linear equation of the standard curve. The sample and control absorbance values were then included in the GlcNAc standard curve equation to obtain the GlcNAc concentration value. One unit of chitinase activity was defined as the number of GlcNAc (µmol) per minute released from the enzymatic reaction under the above conditions.

Optimization of *Bacillus cereus* SMG 1.1 Culture Medium Composition with a Statistical Approach

The optimization stage was carried out by transferring 0.5 ml of inoculum into 100 ml of colloidal chitin broth with various treatments of the medium components. Bacterial cultures were incubated at 30°C in a water bath shaker at 100 rpm for 3 days. At the end of incubation, the chitinase activity was analyzed. The optimization of the medium composition for the production of *B. cereus* SMG 1.1 chitinase was carried out using an experimental design consisting of 2 stages, namely the Plackett-Burman design to select variables that affect the production of *B. cereus* SMG 1.1 chitinase and the Box-Behnken design (response surface method) to optimize the concentration of the selected variable.

Table 1.
Values of the experimental variables for the application of Plackett-Burman Design

Variables (%)	Names	Levels	
		Low (-1)	High (+1)
X_1	K_2HPO_4	0.07	0.14
X_2	KH_2PO_4	0.03	0.06
X_3	Fructose	0.5	1
X_4	Ammonium Nitrate	0.5	1
X_5	Colloidal Chitin	1	2
X_6	$MgSO_4.5H_2O$	0.05	0.1

Source: Data analysis

In the Plackett-Burman design, the variables were divided into 2 groups. The fixed variables were chitinase activity and 6 independent variables consisting of K_2HPO_4 (X_1), KH_2PO_4 (X_2), Fructose (X_3), Ammonium Nitrate (X_4), Colloidal Chitin (X_5), and $MgSO_4.5H_2O$ (X_6). The six independent variables were represented by 2 levels: high (+1) and low (-1) (Table 1). A total of 15 Plackett-Burman experimental designs was determined by the Minitab 14 software. Variables with a degree of confidence above 95% were selected as variables with a significant effect. Significant variables that affect the production of chitinase *B. cereus* SMG 1.1 continued to the optimization stage using the Box-Behnken design to determine the optimal level of the selected variables.

Table 2.
Values of the experimental variables for the application of Box-Behnken Design

Variables (%)	Names	Levels		
		Low (-1)	Medium (0)	High (+1)
X_1	Fructose	0.5	0.75	1
X_2	Colloidal chitin	1	1.5	2
X_3	$MgSO_4.5H_2O$	0.05	0.075	0.1

Source: Data analysis

The selected medium component variables were then tested using 3 different concentration levels, namely high (+1), medium (0), and low (-1) (Table 2). A total of 15 Box-Behnken experimental designs were determined by the Minitab 19 software. The mathematical model used in this study was a polynomial function which aimed to determine the interaction between variables and determine the maximum critical point of the influential variable. The mathematical model is represented in the following equation:

$$y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \sum_{1 \le i \le j}^{k} \beta_{ij} x_i x_j + \varepsilon$$

where y: chitinase activity; k: number of variables; 0: constant; i: coefficient of linear parameter; ii: coefficient of quadratic parameter; ij: coefficient of interaction parameter; xi: variable 1; xj: variable 2; **ε**: Error.

Evaluation of the model's suitability was accomplished by comparing the results of the optimal calculation of the Box-Behnken design with the results of observations with 3 replications. Data analysis was carried out using Minitab 19 software.

RESULTS AND DISCUSSION

Determination of the Medium Components Contributing to Chitinase Production

The receptor-inducer system regulates bacterial chitinase production, which means that the composition of the culture medium and fermentation conditions can substantially affect chitinase production (Patil et al., 2000). In addition, the production of chitinase by bacteria is influenced by bacterial growth. Siboro (2017) showed that the production of chitinase *B. cereus* SMG 1.1 increased along with cell growth and reached the highest value on day-3 when cells reached the late log phase of growth. Therefore, optimizing chitinase production can be accomplished by optimizing bacterial growth factors. The growth medium plays a huge role in providing the nutritional needs for bacteria for growth. Carbon and nitrogen are the primary nutrients in growth. Carbon sources can affect chitinase activity, depending on the suitability of the carbon source to bacterial preferences (Shivalee et al., 2018). Nitrogen is an essential factor in cell metabolism because it affects the synthesis of enzymes, both synthesis in the formation of primary and secondary metabolites. Bacteria also need nitrogen for protein and amino acid synthesis in the growth phase (Sharmistha et al., 2012).

Table 3.
Plackett-Burman experimental design matrix with chitinase activity response

Run	Variables						Chitinase Activity (U/ml)
	K_2HPO_4	KH_2PO_4	Fructose	Ammonium Nitrate	Colloidal Chitin	$MgSO_4.5H_2O$	
1	1	1	1	-1	1	1	0.0022
2	1	1	-1	1	-1	-1	0.0003
3	1	1	-1	1	1	-1	0.0000
4	1	-1	1	-1	-1	-1	0.0000
5	-1	-1	1	1	1	-1	0.0009
6	0	0	0	0	0	0	0.0019
7	-1	-1	-1	1	1	1	0.0009
8	-1	1	1	-1	1	-1	0.0001
9	-1	-1	-1	-1	-1	-1	0.0000
10	1	-1	-1	-1	1	1	0.0001
11	1	-1	1	1	-1	1	0.0000
12	0	0	0	0	0	0	0.0000
13	-1	1	1	1	-1	1	0.0003
14	0	0	0	0	0	0	0.0013
15	-1	1	-1	-1	-1	1	0.0004

Source: Data analysis

The Plackett-Burman experimental design was used to determine the effect of 6 types of medium components as independent variables on the production of chitinase *B. cereus* SMG 1.1. The maximum response of chitinase activity from a total of 15 experimental designs were 0.0022 U/ml (Table 3). Table 4 shows that fructose, colloidal chitin, $MgSO_4.5H_2O$ are the three most influential components in the production of chitinase *B. cereus* SMG 1.1 compared to other components of the medium. It is indicated by the p-value, which is smaller than the other components.

Table 4.
Statistical analysis of Plackett-Burman design for chitinase activity production by *Bacillus cereus* SMG 1.1.

Term	Effect	Coef	SE Coef	T-Value	P-Value	VIF
Constant		0.001078	0.000547	1.97	0.188	
K_2HPO_4	-0.000151	-0.000076	0.000306	-0.25	0.828	1.25
KH_2PO_4	0.000369	0.000185	0.000387	0.48	0.68	2
Fructose	0.00055	0.000275	0.000387	0.71	0.551	2
Ammonium Nitrate	0.000055	0.000028	0.00041	0.07	0.952	2.25
Colloidal Chitin	-0.00079	-0.000397	0.000564	-0.7	0.554	4.25
$MgSO_4.5H_2O$	0.001071	0.000536	0.000612	0.88	0.474	5

Source: Data analysis

Shivalee et al. (2018) stated that fructose has a significant effect on the production of *Streptomyces pratensis* chitinase. The addition of 1.25% fructose produced a maximum chitinase of 0.904 U/ml in *S. pratensis*. Fructose is one of the primary carbon sources that can increase the titer of enzymes in chitinase production by *S. pratensis*. Bacteria use the preferred carbon source based on the CCR (carbon catabolite repression) mechanism (Titgemeyer & Brückner, 2002).

According to Cheba & Zaghloul (2017), colloidal chitin is the most critical component in chitinase production because chitinase is an inducible enzyme. When the colloidal chitin concentration is optimal, maximum chitinase production will be obtained (Bhattacharya et al., 2016). Chitin is a substrate for chitinase, so that the addition of colloidal chitin into the production medium will stimulate the production of chitinase enzymes. Uria et al. (2005) stated that the addition of colloidal chitin increased chitinase activity because of its high solubility in growth media and reduced structural cohesiveness, making it easy for chitinase to degrade colloidal chitin. The maximum chitinase production was obtained by adding 1.5% colloidal chitin to *S. pratensis* (Shivalee et al., 2018). Akhir et al. (2009) found that *B. licheniformis* TH-1 produced the highest chitinase of 1.163 U/ml with 1% colloidal chitin.

Minerals also influence chitinase production. Ghorbel-Bellaaj et al. (2012) stated that $MgSO_4$ has a positive effect on increasing chitinase *B. cereus* SV1. Chitinase production of *Pantoea dispersa* can be increased by adding KBr and $MgSO_4$ (Gohel et al., 2006). $MgSO_4$ also affects chitinase production in *Streptomyces* sp. Da11 (Han et al., 2008). Aounallah et al. (2017) stated that *B. licheniformis* produced a maximum chitinase of 0.5 U/ml with the addition of 0.75% colloidal chitin and 0.01% $MgSO_4$. Thus, fructose, colloidal chitin, and $MgSO_4.5H_2O$ were selected as variables to be continued for the next stage of optimization using the Box-Behnken design.

Optimization of Medium Concentration on Chitinase Production

Three variables selected from the Plackett-Burman design, namely fructose (X_1), colloidal chitin (X_2), and $MgSO_4.5H_2O$ (X_3), were further optimized using the Box-Behnken response surface method and ANOVA analysis to obtain the best concentration. The maximum response of chitinase activity from a total of 15 experimental designs were 0.0016 U/ml (Table 5). The Box-Behnken experimental design is considered a powerful tool to determine the optimal level of relevant factors and their interactions (Ghanem et al., 2010). This design has been used to optimize the culture conditions of various types of bacteria for chitinase production, such as *B. pumilus* (Tasharrofi et al., 2011), *B. licheniformis* AT6 ((Aounallah et al., 2017), and *B. cereus* SV1 (Ghorbel-Bellaaj et al., 2012).

Table 5.
Box-Behnken design matrix with chitinase activity

Run	Variables			Chitinase Activity (U/ml)
	Fructose	Colloidal chitin	$MgSO_4.5H_2O$	
1	-1	0	1	0.0001
2	-1	1	0	0.0003
3	0	1	-1	0.0003
4	0	0	0	0.0003
5	0	-1	-1	0.0002
6	-1	-1	0	0.0004
7	0	1	1	0.0006
8	1	1	0	0.0002
9	0	0	0	0.0016
10	0	-1	1	0.0004
11	1	0	1	0.0003
12	-1	0	-1	0.0002
13	0	0	0	0.0004
14	1	-1	0	0.0002
15	1	0	-1	0.0002

Source: Data analysis

Table 6 indicates an interaction between colloidal chitin and $MgSO_4.5H_2O$ with a p-value of 0.037 (less than 0.05), which indicates that the two variables support each other in optimizing the production of chitinase *B. cereus* SMG 1.1. The lack of a fit value of 0.422 (more than 0.05) indicates that the results of the resulting regression equation are suitable. The quadratic equation resulting from the Box-Behnken analysis is as follows:

$y = 0.001023 - 0.00178\ X_1 + 0.000418\ X_2 - 0.0046\ X_3 + 0.001138\ X_1^2 + 0.000092\ X_2^2 + 0.0724\ X_3^2 - 0.000144\ X_1*X_2 + 0.002916\ X_1*X_3 - 0.002142\ X_2*X_3$

Table 6.
Statistical analysis of Box-Behnken design and significance of the factors and their interaction

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	9	0	0	1.680	0.196
Linear	3	0	0	0.860	0.411
Fructose	0	0	0	0	0.184
Colloidal chitin	1	0	0	0.210	0.910
$MgSO_4.5H_2O$	0	0	0	0	0.613
Square	3	0	0	1.280	0.378
Fructose * Fructose	1	0	0	2.820	0.134
Colloidal chitin * Colloidal chitin	1	0	0	0	0.609
$MgSO_4.5H_2O$ * $MgSO_4.5H_2O$	0	0	0	1.140	0.334
2- Way Interaction	3	0	0	0	0.141
Fructose * Colloidal chitin	1	0	0	0	0.897
Fructose * $MgSO_4.5H_2O$	1	0	0	1.980	0.219

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Colloidal chitin * $MgSO_4.5H_2O$	1	0	0	7.710	0.037
Error	0	0	0		
Lack-of-Fit	0	0	0	1.510	0.422
Pure Error	0	0	0		
Total	0	0			

Source: Data analysis

The interaction between colloidal chitin and $MgSO_4.5H_2O$ has an essential effect on chitinase production by *B. cereus* SMG 1.1. Table 6 shows that the colloidal interaction of chitin and $MgSO_4.5H_2O$ has a significant effect in increasing the production of chitinase *B. cereus* SMG 1.1. These two variables play a mutually supportive role in increasing the production of chitinase B. *cereus* SMG 1.1. Mineral such as magnesium is needed to stabilize ribosomes, membranes, nucleic acids, and the activity of many enzymes (Madigan et al., 2015). Colloidal chitin plays an important role as an inducer in stimulating the formation of the chitinase enzyme. Figure 1 shows that the maximum production of chitinase was obtained at the midpoint, namely 0.75% fructose, 1.5% colloidal chitin, and 0.075% $MgSO_4.5H_2O$.

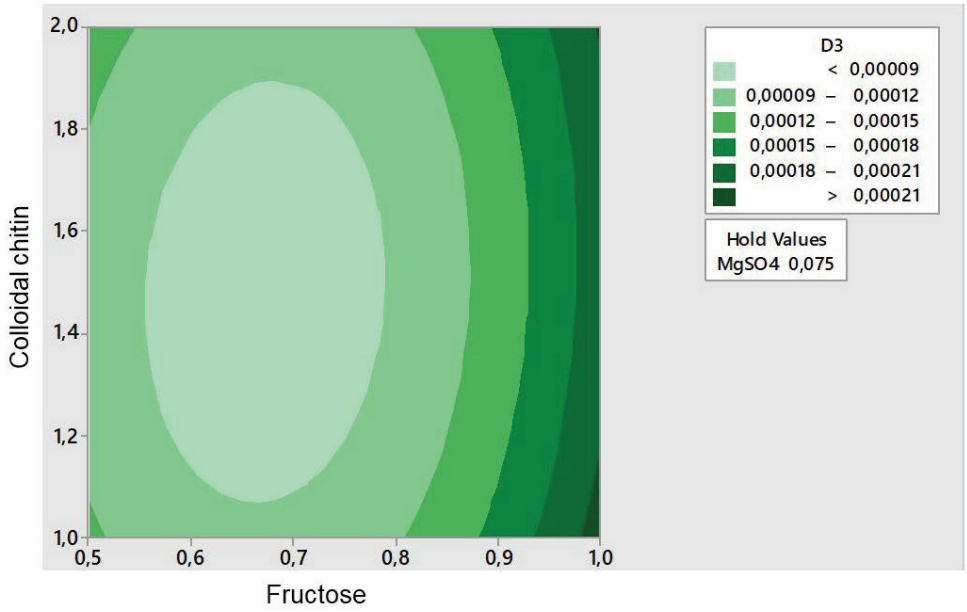

Figure 1.
Plot Contour of the Effect of Chitin and Fructose Colloidal on Chitinase Activity
Source: Data analysis

In verifying the model, the observed chitinase activity was compared with the calculated value from the obtained mathematical model (Table 7). The observed chitinase activity was 0.0009 U/ml, meanwhile the calculated chitinase activity was 0.0010 U/ml, showing a conformity rate of 98%. A level of accuracy above 95% indicates that the model is valid. The highest yield from Box-Behnken was 0.0016 U/ml, an increase of 0.3 times from the previous result of 0.0012 U/ml (Siboro, 2017). These results have not resulted in a significant increase compared to other studies that can increase the results 6.9 times (Ri-

shad et al., 2016) and 10 times (Aounallah et al., 2017) from the initial conditions. It might be caused by other factors that have not been included in the optimization, such as pH, temperature, NaCl, and length of incubation time. Therefore, more factors of growth condition should be included in further optimization of growth condition. Another important note that worth to be pointed out is the low stability of bacterial culture used in this experiment. It was noted from the variation of chitinase activity in the same running condition. This might be happened due to spontaneous mutations that might be present in a sequenced population during the cultivation even in the absence of the exogenous stress factors (Schroeder et al., 2018). Bacterial culture stability is an important issue for industrial bacterial cultures. Therefore, preservation methods of long-term stored stocks culture and production procedure of bacteria culture is very important to be addressed (Wassenaar & Zimmermann, 2020).

Table 7.
Validation of Box-Behnken analysis results

Run	Chitinase Activity (U/ml)	Average observed chitinase activity (U/ml)	Calculated chitinase activity (U/ml)
1	0.0009		
2	0.0007	0.0009	0.0010
3	0.0010		

Source: Data analysis.

CONCLUSION

This study revealed that fructose, colloidal chitin, and $MgSO_4.5H_2O$ are the important medium component for *B. cereus* SMG 1.1 in producing chitinase. The highest yield of chitinase from *B. cereus* SMG 1.1 was achieved by the composition of colloidal chitin broth with 0.75% fructose, 1.5% colloidal chitin, and 0.075% $MgSO_4.5H_2O$. However, the chitinase activity produced in this research is still considered low when compare to other studies. Further optimization with various factors of growth condition such as NaCl, trace minerals, various nitrogen sources is required to increase the production of *B. cereus* SMG 1.1. As the primary metabolite, the production of chitinase is related to the cell growth. Therefore, the measurement of bacterial growth is important to be evaluated to understand the relationship between medium component and bacterial growth for enzyme production.

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