Optimization of Single Cell Protein (SCP) Production using Saccharomyces Cerevisiae by Response Surface Methodology (RSM) on Cell Biomass and Protein Percentage

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ABSTRACT
This study aims to determine the effect of using molasses, KH_2PO_4 and $MgSO_4$ media with different concentrations and formulations on *S. cerevisiae's* growth in manufacturing single-cell protein (SCP). The method used was the response surface methodology (RSM), consisting of 14 treatment formulations and 2 replications. The test parameters carried out in this study were cell biomass and crude protein content. Obtaining data were analyzed using the expert design program 11 with the response surface methodology (RSM) method and the box-Behnken experimental design. The relationship between fixed variables and independent variables of cell biomass is modeled $Y:+6.54-28.63X_1-0.0050X_2-.0112X_30.4025X_1X_2+0.0900X_1X_3-0.0775X_2X_3$, while crude protein is modeled $Y:+6.54-28.63X_1-0.0050X_2-0.0112X_30.4025X_1X_2+0.0900X_1X_3-0.0775X_2X_3$. The results showed that the higher the concentration of molasses, the increased cell biomass. The highest cell biomass value was obtained in the addition of 20% molasses to produce 2.33 grams, while the addition of 20% molasses and 0.7% KH_2PO_4 crude protein produced a low crude protein of 6.00%.

Keywords: *Cell biomass, crude protein, optimization, response surface methodology and single-cell protein.*

1. INTRODUCTION

Protein is an essential building block in the human life cycle. The main protein components are amino acids that function as metabolic processes in the body and are divided into two groups, namely essential and non-essential amino acids [1].

Single Cell Protein (SCP) contains essential amino acids and minerals to be used as food ingredients (for humans) and feed ingredients (for animals). The advantages of Single Cell Protein compared to other protein products are high productivity, fast manufacturing process, easy to obtain substrates and the only use of waste with the help of microbes.

Microbes used as Single Cell Protein producers must have criteria that are not pathogenic, have good nutritional value, can be used as food or feed, do not contain toxic compounds, and have low production costs [2]. PST-producing microbes generally grow in waste containing carbon and nitrogen elements [3]. Several microbes such as bacteria, fungi, algae, and yeasts can produce Single Cell Protein; of the four microbes, the most optimum for PST growth in yeast.

Several studies related to optimization (growth) still use trial and error techniques. This technique takes long without considering the influencing factors, making it less efficient and less controlled [4]. The existence of new methods such as response surface methodology (RSM) can assist in effective optimization, especially in

the development of analytical methods, fractionation extraction, isolation, and others. In this study, the analysis of RSM can produce optimum values of two different variables, such as cell biomass and crude protein. The method in determining cell biomass and crude protein in this study aims to determine which formulation produces the highest cell mass and crude protein because the measurement of protein content is an indirect method of measuring cell biomass based on the measurement of cell components in the form of protein.

2. STYLE PALETTE

2.1 Preparation of Saccharomyces cerevisiae cultures:

Producing a working culture of *S. cerevisiae* by taking 1 ose of *S. cerevisiae* isolate, then put into a microtube containing 1 ml of YPDB media and incubated for 48 hours at a temperature (20-30ºC). The second stage is making 250 ml of media formulated with different concentrations of ingredients using RSM. The third stage is the media sterilization process using an autoclave. The fourth stage was adding 1 ml of *S. cerevisiae* bacterial culture and incubating for 72 hours at 35ºC. The last stage is after the fermentation process, and harvesting is done by centrifugation of the solution, which will produce pellets and dried using an oven at a temperature of 60ºC for 20-25 hours to obtain a single cell protein product (SCP).

2.2 Research design

The research design that will be carried out on the optimization process uses the expert design (DX 11) method of RSM box-Behnken construction consisting of 14 factorial treatments with 2 replications. Box-Behnken is a responsive design with a surface with three factorial design levels to limit the sample size [5]. This design aims to determine the parameters as fixed variables and the sample formulation as independent variables. The fixed variables consisted of cell biomass (Y1) and crude protein (Y2). While the independent variable treatment factors are molasses concentration as factor 1 (X1), KH_2PO_4 concentration as factor 2 (X2) and $MgSO_4$ as factor 3 (X3), whose range and level have been determined in Table 2. Stages to optimize the process of making PST obtained the formulation of the RSM method, and then the independent variables are shown in Table 1.

2.3 Data analysis

The data analysis technique in this study used a design expert 11 programs to optimize a factor. This program has a numerical accuracy of up to 0.001 to determine a suitable mathematical model in the optimization process [6]. A good model has a significant value for the response and has little value for the lack of fit, the adjusted R-squared and predicted R-squared values are supportive. This program can also process four different research designs: collaborative design, factorial design, mixture design, and response surface method design. The fixed variables were analyzed using ANOVA one by one, while the independent variables were analyzed using the lack of fit value. The ANOVA model used is the model that has the highest level and produces significant values. ANOVA model options were shown in the fit summary, such as modified, design model, mean, linear, 2FI, quadratic, cubic, quartic, fifth and sixth. The DX 11 program also provides a standard plot of the residual facility (typical plot of residual), which functions to indicate residuals (difference between the actual response and the predicted response value) and follow the regular line (straight line). If the data points are closer to the normality line indicating customarily distributed data, then the actual results will approach the results predicted by the program [7].

Table 1. *S. cerevisiae* growth formulation for SCP production based on RSM method

Run	Factor 1	Factor 2	Factor 3
	X1:Molase	X2:KH_2PO_4	X3:$MgSO_4$
	%/L	%/L	%/L
1	10	0.55	0.05
2	15	0.4	0.2
3	15	0.55	0.125
4	20	0.55	0.05
5	20	0.55	0.2
6	15	0.7	0.2
7	10	0.7	0.125
8	10	0.4	0.125
9	20	0.7	0.125
10	15	0.55	0.125
11	15	0.4	0.05
12	20	0.4	0.125
13	10	0.55	0.2
14	15	0.7	0.05

Table 2. Determination of independent variables and treatment codes in research

Independent variable	Symbol	Range and Level		
		-1	0	1
		----------%L----------		
Molasses (%)	X1	10	15	20
KH_2PO_4 (%)	X2	0.4	0.55	0.7
$MgSO_4$ (%)	X3	0.05	1.25	0.2

2.3.1 Cell biomass analysis

Cell biomass analysis is the calculation of cell weight using the dry weight method. Before starting the cell biomass calculation, the first step is that the media sample is centrifuged at 3,000 rpm for 15 minutes until the pellet and supernatant are separated. The second stage is taking the precipitate called pellet and filtering it using filter paper, and then the wet cell biomass is transferred to dry filter paper and stored in sterile Petri dishes. The third stage is the drying process using an oven with a temperature of 60°C for 20-25 hours until a constant weight is obtained and dry cell biomass is obtained.

2.3.2 Protein Analysis

Protein parameter testing is determined using the Kjeldahl method, which is still accurate enough to determine protein content, presented in Figure 5. The first stage begins with destroying a sample of 1 gram using a Kjeldahl flask and concentrated sulfuric acid (H_2SO_4) 95-97% as much as 25 ml and adding a catalyst, namely a tablet of Kjeldahl. The destruction results can be seen by changing the color of the sample solution to clear greenishly. The second stage is the distillation process; from the destruction results, it is diluted with 15 ml of distilled water, and 2-3 drops of PP (Phenolphthalein) indicator are added. The third stage is by adding 30% sodium hydroxide (NaOH) and is captured by a solution of boric acid (H_3BO_3) as much as 20 ml, to which 2-3 drops of the MR-BCG indicator have been added in the Erlenmeyer container. The fourth stage is the titration process with 0.1 N hydrochloric acid (HCl) solution until the sample turns bright purple. The volume of HCl used for the titration is recorded and calculated by the following formula:

$$\% \text{ Protein} = \frac{(V2-V1) \text{ x N x } 0.014 \text{ x fk x}}{100\% \text{ W (g)}}$$

W = Sample weight (grams)
V1 = Volume of HCl 0.1 N from blank titration
V2 = Volume of 0.1 N HCl resulting from sample titration
N = Normality HCl
Fk = Conversion factor

3. RESULTS AND DISCUSSION

3.1 Cell Biomass

The results of the analysis of the research parameters of cell biomass and crude protein in the manufacture of Single Cell Protein (SCP) on molasses, KH_2PO_4 and $MgSO_4$ media using Response Surface Methodology (RSM) with different formulations obtained data that can be seen in Table 3.

Models that can describe the relationship between cell biomass and crude protein parameters on optimizing single-cell protein production (SCP) are linear and 2FI models. These results indicate that the suggested or desired model is by the response data [8]. The value of the significance of the model, the lack of fit, and the coefficient of determination (predicted R-squared, adjusted R-squared) indicate a match between the distribution of the data and the model. The model also has a good precision adequacy value (Adeq. Precision>4) of 13.1585 for cell biomass and 12.0305 for crude protein. It indicates that the research has a high level of reliability and accuracy [9]. Based on the two fixed variables and the ANOVA analysis that has been obtained, the model data is presented in Table 4.

The ANOVA results obtained indicate that molasses significantly affected cell biomass response with a p-value of less than 0.05 (<0.0001). The cell biomass response model has an F value of 22.08, indicating that the model is significant, which means it can still be described well by the model and can be used as a predictive model at the optimization stage to get the optimal formula. If the p-value is less than 0.05, then the variable can significantly affect other variables. The smaller the p-value, the greater the level of significance [10].

The appropriate model selected by the program for cell biomass response is a first-order (linear) model with an R^2 value of 0.8689. The R^2 value close to one indicates a high degree of correlation between the observations and the resulting model [11]. In addition to R^2, lack of fit is also used to determine the level of conformity between the data and the resulting model. The lack of fit value obtained has a significant effect because the resulting value is not significant, namely 0.2353 (P> 0.05); this indicates that the linear model is very suitable for estimating fixed variables [12]. The linear model on the yield of cell biomass has the following response equation:

Y: +1.58+0.4837X1+0.1312X2-0.0450X3.

The test results show that the amount of cell biomass weight in each formulation is different; this is caused by several factors that influence it, such as; substrate composition, substrate concentration, fermentation time, temperature and pH [13]. The high substrate used will impact the faster microbial growth, the more overhauled nutrients and the more cell biomass obtained [14].

Table 3. Results of analysis of cell biomass and crude protein on single-cell protein

No.	Molasses	KH_2PO_4	$MgSO_4$	Cell biomass	Crude protein
1	10	0.55	0.05	1.24	7.02
2	15	0.4	0.2	1.54	6.54
3	15	0.55	0.125	1.56	6.25
4	20	0.55	0.05	2.33	6.13
5	20	0.55	0.2	2.21	6.34
6	15	0.7	0.2	1.54	6.37
7	10	0.7	0.125	1.12	7.24
8	10	0.4	0.125	1.04	6.44
9	20	0.7	0.125	2.23	6.00
10	15	0.55	0.125	1.64	6.44
11	15	0.4	0.05	1.42	6.46
12	20	0.4	0.125	1.55	6.81
13	10	0.55	0.2	1.05	6.87
14	15	0.7	0.05	1.71	6.60

Table 4. Analysis of variance based on response surface methodology (RSM) for cell biomass

	Cell biomass			
Source	Predicted Coefficients	Standard Error	Df	P-value
Model	1.58	0.0467	3	<0.0001
X_1	0.4837	0.0618	1	<0.0001
X_2	0.1312	0.0618	1	0.0597
X_3	-0.0450	0.0618	1	0.4834

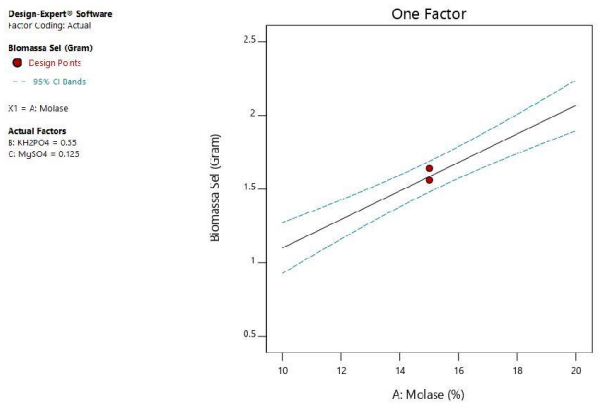

Figure 1. Cell biomass one-factor graphic model

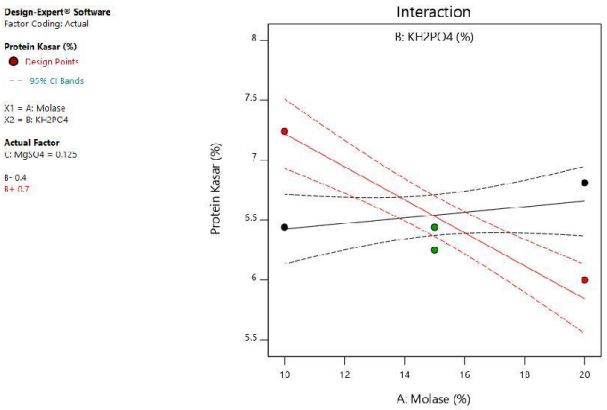

Figure 2. Graph of interaction percentage of crude protein value with molasses and KH_2PO_4

In Figure 1, it can be seen that the average (mean) value produced is 1.58 grams, with the highest cell biomass value of 2.33 grams (treatment of sample formulation number 4) and the lowest value of 1.04 grams (treatment of sample formulation number 8). According to [15], during the fermentation process, *S. cerevisiae* hydrolyzes sucrose into glucose and fructose with the help of the zymmase enzyme, which is used as the primary carbon source was absorbed through an active transfer process. Zymase enzyme functions as a breaker of sucrose into monosaccharides (glucose and fructose) which were then metabolized to produce energy and synthesize cell-forming materials to obtain biomass products [16].

3.2 Protein

Analysis of Variance (ANOVA) test showed that the use of molasses and KH_2PO_4 media had an interaction and significant effect ($P<0.05$) on crude protein content with a p-value of 0.0059, and the resulting response model had an F value of 8.65 (Table 6). According to [17], if the p-value is less than 0.05, it indicates that the model is significant. The model chosen by the program for the appropriate response to crude protein levels is the 2FI model (interaction between 2 factors) with an R^2 value of 0.8812. The value of R^2, which is close to one, can be said that the effect of the fixed variable on the independent variable is significant, so the equation model is suitable to explain the effect of molasses and KH_2PO_4 [18]. In addition to R^2, lack of fit is also used to measure the model's inaccuracy to the observation data obtained at specific probabilities. The lack of fit value obtained has a significant effect because the resulting value is not significant, namely 0.5506 ($P> 0.05$). A slight lack of fit is a requirement for a good model because it shows the suitability of crude protein response data with the model [8]. The model will be considered appropriate when the p-value of the lack of fit is above the probability level used. In other words, the desired p-value of a statistical analysis of lack of fit is not significant. The 2FI model on the percentage of crude protein produced has the following response equation: Y: +6.54-28.63X1-0.0050X2-0.0112X3-0.4025X1X2+0.0900X1X3-0.0775X2X3.

Of the results of the calculation of the percentage of crude protein content using the design expert program, the highest was found in the sample with an average of 6.54%, with the lowest crude protein content of 6% (treatment number 9) and the highest 7.24% (treatment number 7). The interaction graph in Figure 2 shows that the concentration of molasses and KH_2PO_4 in the optimization of SCP production affects the percentage of crude protein value produced. Crude protein value is a calculation through the approach to the total N (nitrogen) content of a material, both from actual protein sources and non-protein nitrogen sources (non-protein nitrogen) [20]. Molasses has a crude protein content of 4.2% [21]. According to [22], the advantages of using molasses are as an additive and have good physical

Table 5. Analysis of variance based on response surface methodology (RSM) for crude protein

	Crude protein			
Source	Predicted Coefficients	Standard Error	Df	P-value
Model	6.54	0.0433	6	0.0059
X_1	-0.2863	0.0573	1	0.0016
X_2	-0.0050	0.0573	1	0.9329
X_3	-0.0112	0.0573	1	0.8498
X_1X_2	-0.4025	0.0810	1	0.0016
X_1X_3	0.0900	0.0810	1	0.3030
X_2X_3	-0.0775	0.0810	1	0.3703

Table 6. The top formula for the correlation solution of the results of the entire response optimum formula recommended by RSM

No.	Molasses	KH_2PO_4	$MgSO_4$	Cell biomass	Crude protein	Desirability
1	14.10	0.65	0.20	1.54	6.55	0.635
2	14.12	0.65	0.20	1.54	6.55	0.635
3	14.06	0.65	0.20	1.53	6.56	0.635
4	14.15	0.65	0.20	1.54	6.55	0.635
5	14.07	0.65	0.20	1.54	6.56	0.635
6	14.07	0.64	0.20	1.53	6.56	0.635
7	14.03	0.65	0.20	1.53	6.56	0.635
8	14.11	0.65	0.20	1.54	6.55	0.635
9	14.01	0.65	0.20	1.53	6.56	0.635
10	14.21	0.65	0.20	1.55	6.54	0.635
11	13.95	0.64	0.20	1.52	6.57	0.635
12	13.95	0.65	0.20	1.53	6.57	0.635
13	13.91	0.65	0.20	1.52	6.57	0.635
14	13.99	0.65	0.20	1.53	6.56	0.635

properties to accelerate the formation of lactic acid and provide available energy sources for *S. cerevisiae* in the fermentation process. Crude protein results obtained indicate that the higher the molasses and KH_2PO_4, the lower the crude protein produced. It was due to the presence of *S. cerevisiae*, which is capable of degrading proteins into peptide bonds and methane gas (NH_3) and compiling them into amino acids, both essential and non-essential [23].

3.3 Correlation of the results of all responses for the optimization stage.

Determination of the optimization response based on the selection of the most suitable model for evaluation on the DX 11 computerized system is in the order of the number of squares (Sequential Model Sum of Squares), model inaccuracy testing (Lack of Fit Tests), and summary statistical models (Model Summary Statistics). Compiling a mathematical model will make it easier for researchers to change the criteria following the expected goals and find out the value of the independent variables that cause the variable values to remain optimal to focus on the desired optimization value. The data on the results of the standard optimization objectives can be seen in Table 5.

The optimum point is determined based on the criteria we want by selecting the interests and desired goals for the response and the existing factors. The desired criteria are cell biomass and crude protein content as maximum as possible. When all the criteria have been selected and determined by the software design expert 11, it will produce the optimum point solution, presented in Table 6.

Table 6 presents the top 14 formulations resulting from the correlation solution of all responses to the optimum formulation recommended by RSM, and only 1 formulation will be selected for the optimization stage. According to [24], it is stated that the accuracy of the formulation and the value of each of these fixed variables can be seen in the desirability value. The desirability value is a parameter that shows the best optimization results with a value range of 0–1 [25]. The closer to one, the solution recommended by the program can meet the wishes according to the criteria that have been set; in other words, the closer to the value of one, the more perfect the desired value. The purpose of optimization is not looking for a value of one but to find the best conditions that bring together all fixed variables in optimum conditions [26].

AUTHORS' CONTRIBUTIONS

All Authors Contributed To The Implementation Of This Research, From The Development Of The Design, The Implementation Of The Research, Data Analysis To The Making Of This Paper.

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REFERENCES

[1] Mandila, S.P., Hidajati, N. (2013). Identifikasi asam amino pada cacing sutra (*tubifexsp*.) yang diekstrak dengan pelarut asam asetat dan asam laktat. Jurnal Kimia, 4(2):97-102.

[2] Adedayo, M.R., Ajiboye, E.A., Akintunde, J.K. and Odaibo A. (2011). Single Cell Proteins: As Nutritional Enhancer, Pelagia Research Library. 2(5): 396-409.

[3] Pawignya, H. (2011). Pembuatan protein sel tunggal dari limbah nanas dengan proses fermentasi. Prosiding Seminar Nasional Teknik Kimia "Kejuangan". ISSN 1693 – 4393.

[4] Telaumbanua, B., Barus, V.T.A., Suryanti, A. (2013). Produktivitas Primer Perifiton di Sungai Naborsahan Sumatera Utara. Program Studi Manajemen Sumberdaya Perairan. Fakultas Pertanian. Universitas Sumatera Utara.

[5] Tekindal. (2012). Box-Behnken Experimental Design in Factorial Experiments: The Importance of Bread for Nutrition and Health. Turkish Journal of Field Crops, 17(2) 2012: hal. 115-123.

[6] Akbar, Adam. (2012). Analisis pengaruh citra merek, harga, dan kualitas produk terhadap keputusan pembelian notebook toshiba. Jurnal Manajemen dan Akuntansi. 12 (2), 14-20. http://publication.gunadarma.ac.id/handle/123456789/6152.

[7] Kumari, V., Cotran, R.S., Robbins, S.L. (2008). Buku Saku Dasar Patologi Penyakit, Ed. 7. EGC, Jakarta, 645 -651.

[8] Keshani, S., Daud, W.R.W., Nourouzi, M.M., Namvar, F. and Ghasemi, M. (2015). Spray drying: An overview on wall deposition, process and modeling. Journal of Food Engineering 146, 152 - 162.

[9] Amin, W.T. (2012). Audit kecurangan dan akuntansi forensik, Harvarindo, Jakarta.

[10] Liu, Z., Thornton, J., Spírek, M. and Butow, RA (2008). Activation of the SPS amino acid-sensing pathway in Saccharomyces cerevisiae correlates with the phosphorylation state of a sensor

component, Ptr3. *Mol Cell Biol* 28(2):551-Journal Article |Research Support, NIH, Extramural Research Support, Non-U.S. Gov't. Standford University. California.

[11] Myers, J.P., Antoniou, M.N., Blumberg, B., Carroll, L., Colborn, T., Everett, L.G., Hansen, M., Landrigan, P.J., Lanphear, B.P., Mesnage, R., Vandenberg, L.N., Vom Saal, F.S., Welshons, W.V. and Benbrook, M.C. (2016). Concerns over use of glyphosate-based herbicides and risks associated with exposures: a consensus statement, Environmental Health, 15:19 DOI 10.1186/s12940-016-0117-0.

[12] Hadiyanto and Suttrisnorhadi. (2016). Response surface optimization of ultrasound-assisted extraction (UAE) of phycocyanin from microalgae Spirulina platensis. Department of Chemical Engineering, Diponegoro University, Jl. Prof. Soedarto, SH-Tembalang, Semarang 50239 Indonesia. Emirates Journal of Food and Agriculture. 2016. 28(4):227-234doi: 10.9755/ejfa.2015-05-193http://www.ejfa.me/.

[13] Nuraini. (2006). Buku Pedoman Bagi PMO (Pengawas Menelan Obat). Semarang : Dinas Kesehatan Provinsi Jawa Tengah.

[14] Winarno, F.G., Fardiaz, S. dan Fardiaz, D. (1980). Pengantar Teknologi Pangan. Gramedia Pustaka Utama, Jakarta.

[15] Hasanah. (2009). Morfologi Kapang dan Khamir. http://hasanah 619.wordpress.com/2009/10/27/ morfologi-kapang-dan-khamir//. (diakses pada September 2021).

[16] Judoamidjojo, R. M., Darwis, A.A. dan Sa‟‟id, E.G. (1992). Teknologi Fermentasi. Bogor: Departemen Pendidikan dan Kebudayaan, Direktorat Jenderal Pendidikan Tinggi, Pusat Antar Universitas Bioteknologi Institut Pertanian Bogor.

[17] Cai, H., Yu, S., Menon, S., Cai, Y., Lazarova, D., Fu, C., Reinisch, K., Hay, J.C. and Ferro, N.S. (2007). TAPPI tethers COPII vesicles by binding the coat subunit Sec23. *Nature* 445(7130):941-4. Journal Article Research Support, NIH, Extramural Research Support, Non-U.S. Gov't. The United States.

[18] Sudjana. (2005). Metode Statistika. Bandung: Tarsito.

[19] Mangunwidjaja D., A.M. Fauzi, Sukardi, Wagiman. (2011). Rekayasa Bioproses Produksi Bioetanol dari Biomassa Lignoselulosa Tanaman Jagung Sebagai Energi Terbarukan. IPB University.

[20] Sukria, H. A. dan Rantan, K. (2009). Sumber dan Ketersediaan Bahan Baku Pakan di Indonesia.IPB Press, Bogor. 53; 58-61.

[21] Juniyanto, M. I., Susilawati, R.I., Supratman, H. (2013). Ketahanan dan kepadatan pellethijauan rumput raja (Pennisetum purpuphoides) dengan penambahan berbagai dosis bahan pakan sumber karbohidrat. Jurnal Universitas Padjadjaran (1-13)

[22] Abidin, Z. (2002). Penggemukan Sapi Potong. Agro Media Pustaka, Jakarta.

[23] Taufik, A., Muzaki, A., Saleh, R. (2017). The Addition of Graphene and Magnetite Materials in TiO2/CuO Catalyst for Enhancing Photosonocatalytic Performance and Reusability. Departemen Fisika, Fakultas MIPA-Universitas Indonesia, Universitas Indonesia, Depok.

[24] Myers, C. L., Chiriac, C. and Troyanskaya, O.G. (2009). Discovering biological networks from diverse functional genomic data. *Methods Mol Biol* 563:157-75. Journal Article. Standford University. California.

[25] Raissi, S. and Farzani, R.E. (2009). Statistical process optimization through the multi-response surface methodology. World Academy of Science, Engineering and Technology. pp. 267–271.