Optimization of waste combinations during decomposition of domestic organic waste using the response surface methodology

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Abstract. Accumulation of domestic organic waste needs to be processed because it can cause negative effects on the environment. Domestic organic waste can be decomposed into high value products of secondary metabolite by using bacteria like biopesticide. Composition of domestic organic waste as a substrate can affect the decomposition of that waste. This research aims to investigate the optimum composition of domestic waste and additional materials in treating domestic organic waste by using Streptomyces sp GMR-22. Composition of the waste and additional materials was determined by proximate nutrition factors of the ash, protein, fat, and carbohydrate contents and optimized by using response surface methodology (RSM). The temperature and moisture was set to 37°C and 60% respectively. The response was demonstrated by the number of Streptomyces sp GMR-22 on the eight day of the solid-state fermentation (SSF) process. The results show that the highest cell number of Streptomyces sp GMR-22 on eight days was resulted from the combination of 5.25 g ash, 5.5 g protein, 1.5 g fat, and 41 g carbohydrate on resulting 2.8 x 10⁹ cells/gram of substrate. The RSM results indicated that carbohydrate and protein contents were the main factors on the growth of Streptomyces sp. GMR-22. On the other hand, ash had a low significance and fat had no significant effect. In the processing of domestic organic waste through SSF which was based on the proximate value, the RSM results can be used to determine nutrition priorities that must be met.

1. Introduction
Organic waste is defined as residual material of natural biodegradation process, as well as waste produced by plants and animals. Most of organic waste comes from household food, agricultural, industrial, and animal waste. Based on data from the Ministry of Environment, solid waste in Indonesia reached 65.8 million tons during 2017, most of them were organic waste. The waste management is varied into: 68% transported and stockpiled, 9% buried, 6% processed into compost and recycled, 5% burned, and 7% unmanaged.
Improper waste management can have a negative impact on the environment and human health. Waste dumps cause leachate emissions that contain organic pollutants, nitrogen, greenhouse gases and garbage-borne diseases, while combustion can cause air pollution [1]. In addition, there are ways that are friendly to the environment such as composting process. Composting of organic domestic waste is done to reduce the toxicity of the waste and increase the available elements for plants. The composting process continues to develop today, but still it cannot overcome the piles of organic waste in the landfill. Composting can be done on a household scale to reduce the waste pile in the landfill, but this is still difficult to do if there is no regulation from the government that regulates it [2].

Another alternative of processing organic waste is the use of solid-state fermentation (SSF) that can be a solution to the problem of domestic waste and the low value of output products. SSF bioreactors are commonly used to produce industrial raw materials with homogeneous organic waste as raw material. The SSF process can produce enzymes, surfactants, biofuels, flavor, and other metabolites that are useful as raw materials in the chemical and pharmaceutical industries or can be directly applied as animal feed and biopesticides that can increase the value of processed waste products [3-6].

Municipal waste includes domestic household and market wastes containing high concentrations of lignocellulose which can be converted into valuable bio-products. However, nutrition in waste is often found under sub-optimal or insufficient conditions that requires pre-treatment and optimization before being processed by using SSF. Response surface methodology (RSM) is an experimental method that can be used to optimize the parameters of the fermentation process by applying mathematics and statistics [7,8] RSM has been adopted to increase the production of antibiotic compounds in several Streptomyces species including Streptomyces sp. 1-14 [9], Streptomyces marinensis [10], and Streptomyces fradiae [11].

Streptomyces is a gram-negative, filamentous, and saprophytic bacterium commonly found in soil, root areas, and compost. These bacteria have the ability to produce hydrolytic enzymes that play role in the degradation of complex organic compounds such as cellulose and production of various kinds of secondary metabolite including antibiotics that are useful in the industrial, medical or agricultural fields [12,13]. In this study Streptomyces sp. GMR-22 was used to produce bio-fungicide compounds [14] and the box-behnken design was used to determine the optimal conditions of the fermentation process. Optimization factors are nutrition (by proximate test), temperature, and humidity. The optimization results will then be applied to a bioreactor to treat domestic waste in the future studies.

2. Methodology

2.1. Domestic waste sample collection
Domestic waste was collected from several markets in the Special Region of Yogyakarta, namely the Kranggan market, Triwindu market, Demangan market, Lempuyangan market, and Giwangan market. The collected waste was dried. A proximate test, then, was carried out to determine the waste content.

2.2. Microorganism and inoculum preparation
Streptomyces sp. GMR-22 was obtained from the Microbiology Laboratory of the UGM’s Faculty of Agriculture. Streptomyces sp. GMR-22 inoculum was prepared by dissolving 5 ml of the spore suspension from the slant agar which had been grown on TSA media for 8 days into a 500 ml Erlenmeyer containing 100 ml TSB media. The inoculum was incubated for 2 days on a rotary shaker at 37 °C.

2.3. Solid-state fermentation
SSF was performed on a 500 ml Erlenmeyer with 42.5 - 54 grams of waste. Nutrient optimization on the growth of Streptomyces sp. GMR-22 was studied by adding agricultural waste which had a proximate stable test result in domestic organic waste according to the proximate composition. Before inoculation and incubation was carried out, the feed was sterilized in the autoclave with a temperature of 121°C for 15 minutes. Each Erlenmeyer was then inoculated with 10 ml of spore suspension and incubated at 37°C for 8 days.
2.4. *Streptomyces* Growth

The growth of *Streptomyces* sp. GMR 22 was measured using a haemocytometer [15]. Solid substrate containing *Streptomyces* sp. GMR-22 spores dissolved in sterile tween liquid. The dissolved spores were then calculated using haemacytometer under a light microscope with 40x ratio.

2.5. Box-Behnken design and response surface analysis.

Response surface methodology was analyzed with Minitab software. The design of the experiment used was box-behnken design. After the response had been determined for each experiment, each response was placed into a second-order polynomial model.

\[
Y = \beta_0 + \sum \beta_i x_i + \sum \beta_{ii} x_i x_j + \sum \beta_{ij} x_i x_j
\]  

(1)

Where \(Y\) is the predicted response bacterial growth, \(B_0\) is a constant coefficient, \(B_i\) is the primary coefficient, and \(B_{ij}\) is the quadratic coefficient, and \(X_i\) is the independent variable.

3. Result and Discussion

Domestic organic waste was characterized by a proximate test where the proximate test factors were determined as nutritional factors which composition will be optimized. As shown in table 3.1, domestic waste taken from various markets in Special Region of Yogyakarta had a varied proximate value composition, while agricultural waste (table 3.2) tended to have a stable proximate composition that enabled it to be used in balancing the nutrient value in domestic organic waste. In order to adjust the content of domestic organic waste, agricultural waste can be categorized into four categories: high carbohydrate content (Cassava lees), high protein content (Wheat bran and corn bran), high fat content (rice bran), and high ash content (Straw).

| Tabel 1. Composition of Domestic Organic Wastes from different markets |
|---|---|---|---|---|---|
| Domestic Organic Waste | Moisture (%) | Protein (%) | Fat (%) | Carbohydrate (%) | Ash (%) |
| Tri Windu Market | 12,59 | 18,19 | 6,90 | 57,50 | 17,3 |
| Demangan Market | 9,085 | 8,98 | 0,65 | 75,31 | 15,04 |
| Lempuyangan Market | 15,35 | 11,28 | 0,77 | 58,44 | 29,49 |
| Kranggan Market | 7,75 | 10,96 | 18,51 | 34,27 | 36,25 |
| Giwangan Market | 10,3 | 15,39 | 1,44 | 54,63 | 28,52 |

| Tabel 2. Composition of different Agricultural Wastes |
|---|---|---|---|---|---|
| Domestic Organic Waste | Moisture (%) | Protein (%) | Fat (%) | Carbohydrate (%) | Ash (%) |
| Cassava lees | 14,5 | 2,14 | 0,22 | 96,21 | 1,40 |
| Rice bran | 9,44 | 5,62 | 7,02 | 71,60 | 15,74 |
| Corn bran | 11,3 | 11,80 | 3,07 | 80,71 | 4,39 |
| Wheat bran | 11,54 | 13,87 | 4,65 | 76,38 | 5,08 |
| Straw | 10,46 | 3,10 | 0,32 | 77,58 | 18,97 |

Previous studies had examined the effect of waste combinations on waste decomposition and glucoamylase production. Although the study analyzed the nutrient value of waste, the combination and optimization was not based on the nutrient value, rather, the combination was based on the weight of each waste [16]. In this study, the nutrient value of agricultural waste was used to regulate the composition of carbohydrate, protein, ash, and fat contents in the domestic organic waste.
The waste was then optimized to get highest cell number of *Streptomyces* sp GMR-22. *Streptomyces* sp GMR-22 that was used in this study was a superior isolate producing antibiotics that can inhibit the growth of *Fusarium oxysporum*, *Candida albicans*, and *Aspergillus flavus* [14]. In addition *Streptomyces* sp. GMR-22 has been studied to have 63 gene clusters that are associated with secondary metabolites, where cluster 41 is defferomite B cluster that has the potential to be anticancer in the cells of colon cancer [17,18].

In order to determine each nutritional factor and its combination, 27 trials were designed using box-behnken design where the response was shown by bacterial growth. The combination of experiments and responses is shown in table 3.3. The highest response was resulted from the combination of 5.25 g ash, 5.5 g protein, 1.5 g fat, and 41 g carbohydrate with the number of colon cancer metabolites, where cluster 41 is defferomite B cluster that has the potential to be anticancer in the cells of colon cancer [17,18].

\[
Y = 15669 + 166,0 X_1 - 479,5 X_2 - 7 X_3 - 806,4 X_4 + 0,17 X_1^2 + 16,85 X_2^2 + 4,59 X_3^2 + 10,425 X_2^2 - 1,30 X_{12} + 0,19 X_{13} - 4,00 X_{14} + 0,88 X_{23} + 10,27 X_{24} - 0,32 X_{34}
\]

Where Y is predicted response (*Streptomyces* sp. GMR-22 growth), X1, X2, X3, and X4 are independent variable (ash, protein, fat, and carbohydrate), X1^2, X2^2, X3^2, X1^2, X2^2, X3^2, X12, X13, X14, X23, X24, and X34 are the quadratic and linear interaction.

**Table 3.** Box-behnken design with observed and predicted response of *Streptomyces* sp. GMR-22 growth.

| Run | X1  | X2  | X3  | X4  | Observed | Predicted |
|-----|-----|-----|-----|-----|----------|-----------|
| 1   | 2,50| 3,5 | 2,5 | 38  | 0,02     | -12,735   |
| 2   | 5,25| 5,5 | 1,5 | 41  | 285,00   | 284,677   |
| 3   | 5,25| 3,5 | 1,5 | 38  | 14,50    | 14,473    |
| 4   | 8,00| 3,5 | 0,5 | 38  | 47,80    | 52,140    |
| 5   | 2,50| 3,5 | 0,5 | 38  | 1,00     | -9,610    |
| 6   | 5,25| 1,5 | 1,5 | 35  | 197,90   | 189,809   |
| 7   | 8,00| 3,5 | 2,5 | 38  | 48,90    | 51,095    |
| 8   | 5,25| 5,5 | 1,5 | 35  | 168,50   | 159,384   |
| 9   | 5,25| 1,5 | 1,5 | 41  | 67,90    | 68,602    |
| 10  | 5,25| 3,5 | 0,5 | 35  | 131,04   | 111,834   |
| 11  | 2,50| 5,5 | 1,5 | 38  | 122,08   | 105,240   |
| 12  | 5,25| 3,5 | 0,5 | 41  | 116,21   | 115,827   |
| 13  | 8,00| 1,5 | 1,5 | 38  | 67,89    | 75,205    |
| 14  | 2,50| 1,5 | 1,5 | 38  | 12,07    | -1,925    |
| 15  | 5,25| 3,5 | 2,5 | 35  | 120,84   | 111,699   |
| 16  | 5,25| 3,5 | 1,5 | 38  | 14,55    | 14,373    |
| 17  | 5,25| 3,5 | 2,5 | 41  | 102,11   | 111,792   |
| 18  | 8,00| 5,5 | 1,5 | 38  | 149,22   | 153,690   |
| 19  | 2,50| 3,5 | 1,5 | 41  | 98,82    | 112,111   |
| 20  | 8,00| 3,5 | 1,5 | 41  | 131,83   | 108,861   |
| 21  | 5,25| 5,5 | 2,5 | 38  | 126,56   | 133,504   |
| 22  | 5,25| 1,5 | 2,5 | 38  | 34,09    | 37,164    |
| 23  | 5,25| 3,5 | 1,5 | 38  | 14,07    | 14,373    |
| 24  | 8,00| 3,5 | 1,5 | 35  | 168,21   | 172,858   |
| 25  | 5,25| 1,5 | 0,5 | 38  | 31,77    | 42,764    |
| 26  | 5,25| 5,5 | 0,5 | 38  | 117,21   | 132,074   |
| 27  | 2,50| 3,5 | 1,5 | 35  | 3,12     | 44,028    |
Figure 1. The observed (x axis) vs predicted (y axis) value of *Streptomyces* sp. GMR-22 growth.

The statistical significance of the regression model generated F value = 23.01 and P < 0.0001. P value that was close to the limit level indicating that the regression equation can be accepted. The prediction values and experimental results of *Streptomyces* sp. GMR-22 growth were then re-tested and the result showed in Figure 3.1. $R^2$ value of 0.967 indicating that the model had 96.7% variability in the response. It also showed that the model can be trusted to predict the value of bacterial growth accurately.

**Table 4.** ANOVA for box-behnken design as a function of ash ($X_1$), Protein ($X_2$), Fat ($X_3$), and carbohydrate ($X_4$).

| Source        | DF | Adj SS | Adj MS | F-Value | P-Value |
|---------------|----|--------|--------|---------|---------|
| Model         | 16 | 128087 | 8005.5 | 23.01   | 0.000   |
| Blocks        | 2  | 866    | 433.2  | 1.25    | 0.329   |
| Linear        | 4  | 74373  | 18593.4| 53.43   | 0.000   |
| $X_1$         | 1  | 3504   | 3504.2 | 10.07   | 0.010   |
| $X_2$         | 1  | 25168  | 25167.7| 72.33   | 0.000   |
| $X_3$         | 1  | 0      | 0.5    | 0.00    | 0.972   |
| $X_4$         | 1  | 47799  | 47799.4| 137.37  | 0.000   |
| Square        | 4  | 69743  | 1743.8 | 50.11   | 0.000   |
| $X_1*X_1$     | 1  | 9      | 8.5    | 0.02    | 0.879   |
| $X_1*X_2$     | 1  | 24240  | 24240.0| 69.66   | 0.000   |
| $X_1*X_3$     | 1  | 112    | 112.2  | 0.32    | 0.583   |
| $X_1*X_4$     | 1  | 46953  | 46952.9| 134.93  | 0.000   |
| 2-Way Interaction | 6  | 19775  | 3295.8 | 9.47    | 0.001   |
Figure 2. Surface plots (3D) and contour plots (2D) showing the interactive effects of the most significant variables (carbohydrate and protein contents) on the growth of *Streptomyces* sp. GMR-22.

The statistical significance of each waste nutrient showed a significant linear effect on $X_1$, $X_2$, and $X_4$ with the P value less than 0.05. P value of $X_3$ that was more than 0.05 indicated a non-significant
linear effect on *Streptomyces* sp. GMR-22 growth. The result indicated that carbohydrate and protein contents were nutritional factors that had the most influence on the growth of *Streptomyces* sp. GMR-22. It was also shown in the values of \( X_{12} \) and \( X_{22} \) and the interaction values of the two components which indicated high significance. Then \( X_1 \), which showed significant values individually, showed insignificant interaction and quadratic values. This showed a low significance of the effect of the ash component on the growth of *Streptomyces* sp. GMR-22 on SSF. This is in accordance with the components of bacterial cells which are generally dominated by carbon, then nitrogen, while ash contains micro elements (P, K, Ca, S, Cu, Mn, Zn, Ni, Cr, Pb, As) [19-21].

In domestic organic waste processing, the regulation of waste nutritional value cannot be done easily to get the optimum value because of the dynamic nutritional value of domestic waste, while agricultural waste that was used as regulator had several nutrients value at once. The RSM results can be used to determine nutrition priorities that must be met. Thus, the composition of waste can be calculated which approaches the optimum value of the growth of *Streptomyces* sp GMR-22 even though the waste used varies as domestic waste.

4. Conclusion

RSM can be used to optimize to optimize the growth of *Streptomyces* sp GMR-22. The highest number of *Streptomyces* sp GMR-22 on eight days was resulted from the combination of 5.25 g ash, 5.5 g protein, 1.5 g fat, and 41 g carbohydrate on resulting \( 2.8 \times 10^5 \) cells/gram of substrate. The RSM results also indicated that carbohydrate and protein were nutritional factors that had the most influence on the growth of *Streptomyces* sp. GMR-22. On the other hand, ash had a low significance and fat had no significant effect. In the processing of domestic organic waste through SSF which was based on the proximate value, the RSM results can be used to determine nutrition priorities that must be met.

Acknowledgements

The authors gratefully acknowledge the waste preparation support by PT Biotek Cipta Kreasi.

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