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Research Article
Optimization of Biogas Production from Rice Husk Waste by Solid State Anaerobic Digestion (SSAD) Using Response Surface Methodology

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Abstract
Background and Objective: Rice husk is one of the agricultural waste which is abundantly available in Indonesia. Due to high content of cellulose, rice husk can be considered as source of biogas substrate after pretreatment of lignin removal. Lignin content can inhibit the production of biogas because microorganisms hard to degrade the rice husks. The aim of the study was to evaluate the effect of pre-treatment by using NaOH and to determine optimum process conditions of biogas production by solid state anaerobic digestion (SSAD) using response surface optimization method. **Materials and Methods:** This study was conducted in a laboratory scale. Enzyme variables were set at 3-9% concentration, carbon/nitrogen (C/N) ratio at 20-50 and total solid (TS) concentration at 15-40%. To determine the daily productivity, biogas production was measured by water displacement method every 2 days for 90 days. A central composite design was employed to set the number of experimental run. Three independent experimental variables, namely, enzyme (X^1), C/N ratio (X_2) and total solid (X_3) were selected as controlled factors. This research also studied the biogas production kinetics constants of biogas production rate (U), maximum biogas production (A) and minimum time of biogas (λ) which were determined by using non-linear regression technique from Gompertz model. **Results:** Based on experiment, biogas produced from rice husk treated with NaOH 3% was higher than the biogas without treatment with the yield of 63.9 mL g^{-1} TS. The optimization showed that the optimal biogas production was achieved at addition of 4.5-7% enzyme and carbon/nitrogen (C/N) ratio of 35-45. The total solid (TS) was not determinant factor in biogas production. **Conclusion:** Pre-treatment using NaOH could increase biogas production significantly. The best biogas production was achieved at 6% enzyme addition, C/N ratio = 35% and total solid of 27.5% with yield of 63.93 mL g^{-1} TS.

Key words: Biogas production, rice husk, gompertz, SSAD, response surface methodology

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Competing Interest: The author has declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Nowadays, energy is one of three important issues in Indonesia including water and food security. The rapid growth of population in Indonesia as well as un controlled urbanization has been also considered as serious cause of energy requirements. To overcome these problems, Indonesia as agricultural country intensively used agricultural waste as source of biomass energy. Currently, the use of biomass as energy is only less than 5%[1].

Digestion under absence of oxygen has been recognized as a proven technology for converting waste to energy through decomposition of organic materials. The product of the anaerobic digestion is biogas which mainly consists of methane (50-60%) and CO_2 (30-40%). The source of organic material can be derived from agricultural waste, livestock or animal waste as long as they have certain C/N ratio. The anaerobic digestion provides alternative technology of generating energy and also waste treatment[2,3]. Anaerobic digestion can be classified based on the total solid content of waste substrate. Liquid anaerobic digestion (LAD) is typical biogas production process by using waste substrate containing less than 15% total solid, while solid state anaerobic digestion (SSAD) utilizes waste substrate with more than 15% total solid[4-8]. The L-AD is suitable for production of biogas from high content water such as sewage and food waste[4,8]. However, this method is less applicable for high cellulose substrate in which require low water content during its fermentation[8,9]. Moreover, compared to L-AD, the SS-AD method has higher productivity of biogas and low energy requirements[10,11].

Rice husk is abundantly available in Indonesia especially in rural area. The main content of rice husk is cellulose (59%), hemi-cellulose (19%), lignin (20%) and ash (1%)[4]. The high content of cellulose, making rice husk is excellent potential for anaerobic digestion. However, it has high lignin which will disturb the decomposition by micro-organism. Therefore, the pre-treatment is required to remove the lignin content from the rice husk.

Biogas productivity has been influenced by some parameters such as temperature, pH, alkalinity, F/M ratio and C/N ratio. The optimization of these parameters in order to search the best productivity have been done by many researchers in batch conventional anaerobic digestion, however to our best knowledge there was no optimization of solid state anaerobic digestion (SSAD) from rice husk by varying the enzyme, C/N ratio and total solid (TS) as variables. To determine correlation these variables with the response therefore Response Surface Methodology (RSM) is a suitable method. Response surface methodology is a statistical method that time and cost-effective tools to describe influences and interactions of input variables to the responses. The aim of this research was to evaluate the effect of chemical pre-treatment using NaOH and to determine the optimum of enzyme, C/N ratio and total solid (TS) content to biogas production in SSAD by using Response Surface Methodology.

MATERIALS AND METHODS

Study period and location: This study was conducted in 2017-2018 at Environmental Laboratory, Department of Environmental Engineering, Universitas Diponegoro Indonesia.

Materials: Materials used in this research include: rice husk obtained from agricultural land in Rowosari, Tembalang, Central Java while rumen cow fluid as inoculum was obtained from slaughter house in Pedurungan, Semarang. The chemical pre-treatment NaOH 3% was used according the method proposed by Chandra *et al.*[12]. The enzyme used was lignase to accelerate the biochemical process.

Experimental set up: Biogas production was carried out in a laboratory biodigester (600 mL). Biodigester was constructed from a polyethylene bottle equipped with a tight rubber plug and fitted with a valve on the top of biodigester to measure the biogas. Biogas was measured by the 'liquid displacement method'[6,13-16]. Schematic diagram of biogas measurement in the laboratory can be seen in Fig. 1.

Biogas production: Prior to biogas production, the rice husk was pre-treated by using NaOH 3% in order to remove the lignin. After pre-treatment, enzyme, C/N ratio and total solid content were adjusted according to experimental design (central composite design) at the variation of 3-6% enzyme concentration, C/N ratio of 20-50 and total solid of 15-40%. The substrate was mixed with inoculum and water according to the TS concentration as well as the technical urea to adjust the C/N ratio variation and the enzyme was added accordingly. The prepared substrate was fed into the bioreactor, sealed tightly for anaerobic conditions and ready for operation. The morphological structure of rice husk before and after pre-treatment was evaluated using Scanning Electron Microscope (SEM). The biogas volume was monitored by passing the gas into a water-filled measuring cup according to displacement method 2 and monitored every 2 days.

Gompertz equation: The accumulated biogas was monitored and recorded. The experimental data was fitted to the

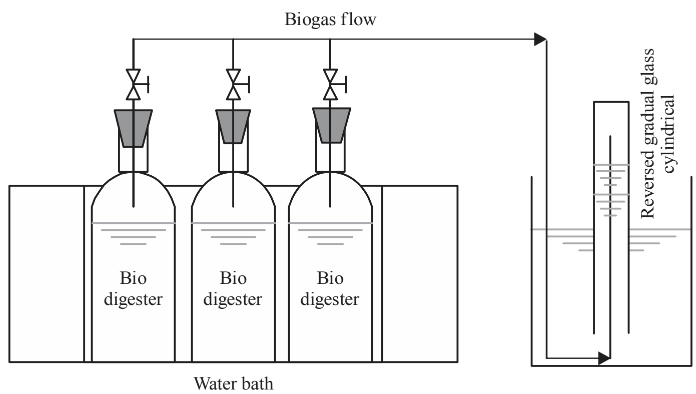

Fig. 1: Schematic diagram of biogas measurement in laboratory[2]

Table 1: Factorial of central composite experimental design for optimizing enzyme, C/N ratio and TS content on biogas production

	Coded variable (x)			Real variable (X)			Yields	
Runs	X_1	X_3	X_3	Enzyme (%)	C/N	TS (%)	Experimental values (mL g^{-1} TS)	Predicted values (mL g^{-1} TS)
1	-1.0	-1.0	-1.0	3.00	20.00	15.00	24.42	27.95
2	-1.0	-1.0	1.0	3.00	20.00	40.00	5.68	5.74
3	-1.0	1.0	-1.0	3.00	50.00	15.00	52.08	64.62
4	-1.0	1.0	1.0	3.00	50.00	40.00	15.06	45.17
5	1.0	-1.0	-1.0	9.00	20.00	15.00	25.30	29.89
6	1.0	-1.0	1.0	9.00	20.00	40.00	6.77	7.63
7	1.0	1.0	-1.0	9.00	50.00	15.00	39.54	75.12
8	1.0	1.0	1.0	9.00	50.00	40.00	12.33	37.65
9	0.0	0.0	0.0	6.00	35.00	27.50	59.21	63.93
10	-1.8	0.0	0.0	0.71	35.00	27.50	15.96	18.97
11	1.8	0.0	0.0	11.29	35.00	27.50	10.51	12.53
12	0.0	-1.8	0.0	6.00	08.54	27.50	8.30	8.83
13	0.0	1.8	0.0	6.00	61.46	27.50	8.85	9.55
14	0.0	0.0	-1.8	6.00	35.00	5.45	108.41	122.10
15	0.0	0.0	1.8	6.00	35.00	49.55	6.72	7.21
16	0.0	0.0	0.0	6.00	35.00	27.50	49.73	52.56

common biogas production model of Gompertz. It is a mathematical model for time series observation, i.e., the slowest growth at the beginning (lag time) and steady after exponential period. Lag period is defined as the time required for microorganism to adapt to their environment before they achieve the exponential period (Eq. 1):

$$P = A.\exp\left\{-\exp\left[\frac{Ue}{A}(\lambda - t)+1\right]\right\} \qquad (1)$$

Where:

A = Growth at lower asymptote
U = Maximum specific growth rate
λ = Lag time (days)
e = Exponent (2.718281828) and
t = Sampling time (days)

The parameters of U, A, λ was determined by non-linear regression of Eq. 1 to experimental data.

Response surface methodology: A central composite design (CCD) with three independent experimental variables, namely, enzyme (X_1), C/N ratio (X_2) and TS content (X_3) and Yi as yield were used to set number of experiments (16 run). The real value of enzyme, C/N ratio and total solid were transformed to encoded variables based on lower, upper and centre points i.e., -1, 1, 0 and α, where +1 denotes high level, -1 low level, α = 2 n/4 (n = number of variables or factors) is the star point and 0 corresponds to the centre point. The dependent variables and independent variables are linked in a polynomial equation (Eq. 2), where the parameters are were obtained based on linear regression[17,18]. Table 1 showed the predicted and observed values of biogas yield calculated by using Eq. 2:

$$Y_i = 54.1600 - 3.2224x_1 + 8.1312x_2 - 39.4985x_3 - 4.3148x_1x_2 + 2.5052x_1x_3 - 6.7367x_2x_3 - 28.0793x_1^2 - 31.0749x_2^2 + 0.4170x_3^2 \quad (2)$$

For statistical calculations, the variables X_i (the real value of an independent variable) were coded as x_i (dimensionless value of independent variable) according to the following Eq. 3:

$$x_i = \frac{(X_i - X)}{\delta} \quad (3)$$

where, Xo is the value of Xi at the center point and dX represents the step change. The correlation between coded and real value of variables are shown in Table 1.

Statistical analysis: The statistical one-way analysis of variance (ANOVA) was used to evaluate the adequacy of the model. The statistical significance of the second-order polynomial of Eq. 2 was checked by an F-test (ANOVA). The Statistica 6.0 software was used to analyze experimental data and to calculate the predicted response by using Eq. 2. The predicted and experimental responses were presented in 2-D or 3-D plots. The significance evaluation between the mean of yield of biogas in different experimental runs were statistically defined at $p<0.05$.

RESULTS AND DISCUSSION

Rice husk characteristic: The characterization study showed that rice husk contains high cellulose contents (58%) and other main compound of lignocelluloses (Table 2). This high content of cellulose is potent for biogas production as source of carbon during methanogenesis. However, the lignin still present in the rice husk material (20%) which inhibit the work of microorganism if it is not removed from the husk. Other part of rice mill waste, rice straw also contains of 36% cellulose and 16% lignin. As other agricultural waste, the lignocellulose contents of cellulose is important source for producing useful products, such as sugars from the fermentation process, chemicals and liquid fuels[19]. Thus, to produce biogas from rice husk waste, there is a need for special pre- treatment prior to the fermentation process in the digester[20,21].

Influence of chemical pre-treatment on biogas production: This study shows the significant increase of productivity of biogas due to the use of pre-treatment of rice husk using NaOH 3%. The increase of productivity was almost 5 times higher than the one without pre-treatment after 90 days of experiment (Fig. 2). The production of biogas was conducted at total solid (TS) of 27.5% and the C/N ratio of 35.

Table 2: Lignocellulosic components of rice waste

Biomass	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Ash (%)
Rice husk	58.852	18.03	20.9	0.16-1
Rice straw	28-36	23-28	16-Dec	15-20

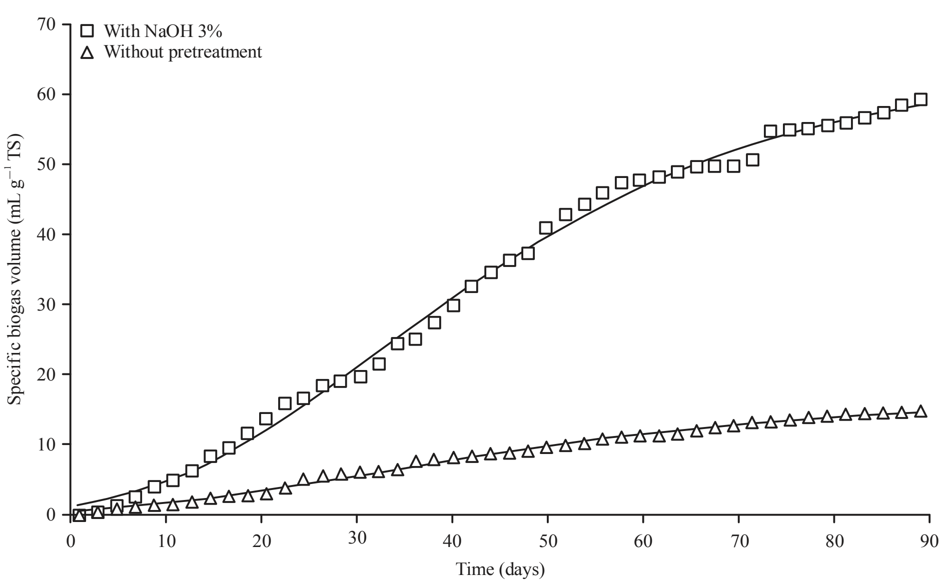

Fig. 2: Relationship between experimental data and model, on the effect of NaOH on biogas production at total solid of 27.5% and C/N ratio of 35

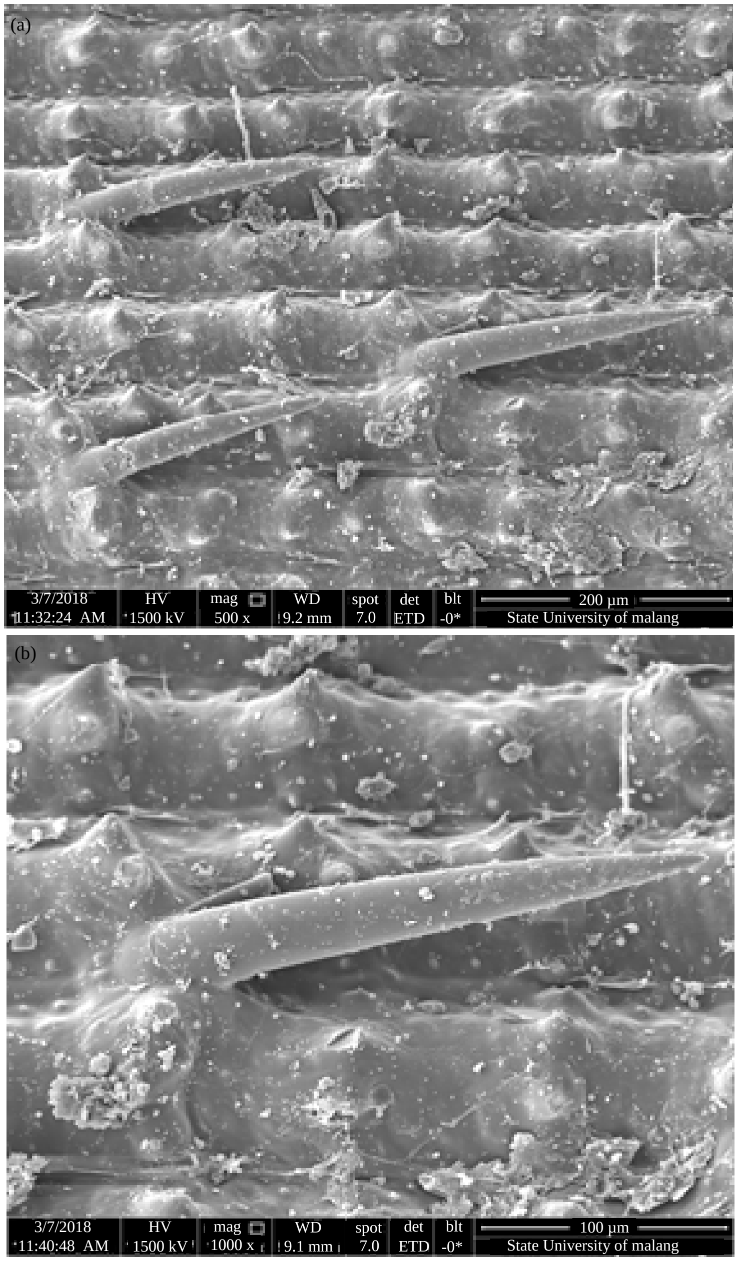

Fig. 3(a-b): SEM of rice husk before addition of 3% NaOH, 500x and 1,000x magnification

According to Gompertz model (Eq. 1), the obtained parameters are maximum biogas production (A) by using pretreatment was 63.9 mL g^{-1} TS with lag time (λ) of 8.29 days and specific growth rate (U) of 1.15/day, while without pretreatment could achieved maximum yield of biogas (A) = 16.4 mL g^{-1} TS with lag time (λ) of 4.6 days and specific growth rate (U) of 0.26/day. The result of this study showed a higher productivity than previous study conducted by Carrillo *et al.*[22] using wheat straw as substrate and pretreated by using NaOH. The similar finding has been shown by Chandra *et al.*[12] by using rice straw. However, they achieved 132% yield after 120 h treatment in a liquid anaerobic digestion. The application of NaOH as pre-treatment agent to remove lignin has been also shown by Taherdanak and Zilouei[23] using wheat straw and 8% NaOH solution.

Morphological structure of treated rice husk: The scanning electron microscope (SEM) has been used to evaluate morphological structure of rice husk before and after treatment. The morphological study showed that the pre-treatment using NaOH has significant destruction to the material (Fig. 3, 4).

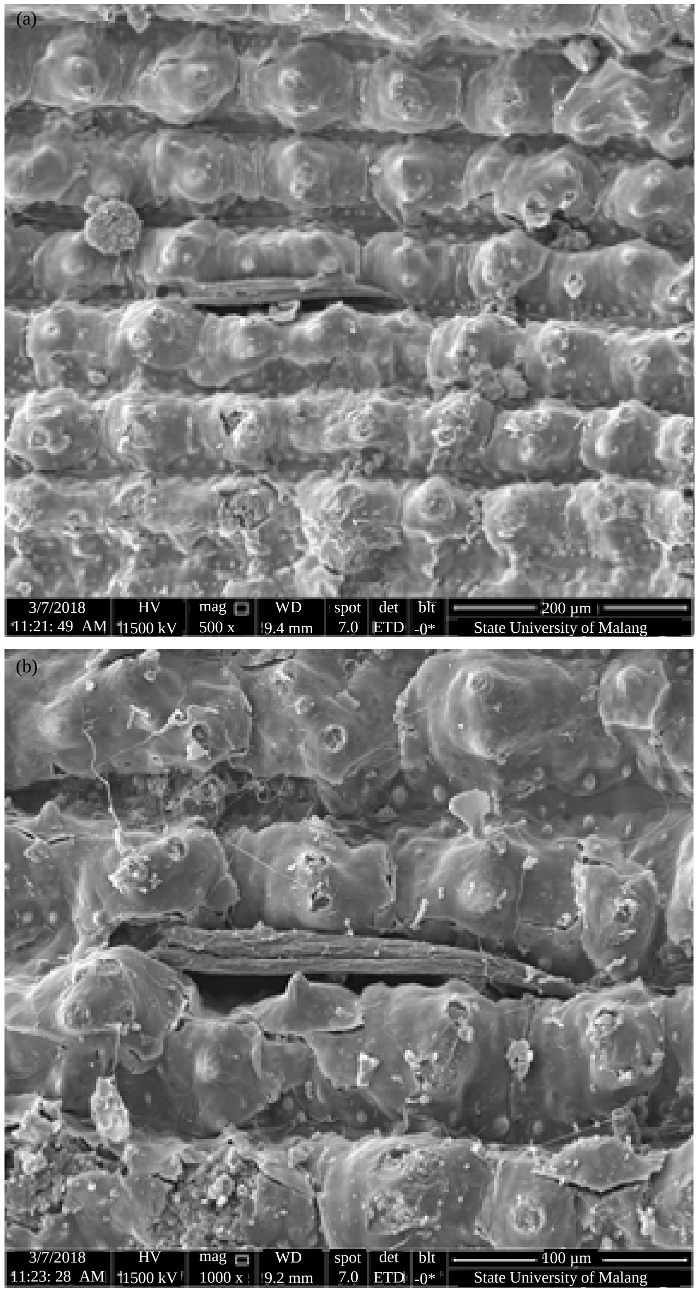

Fig. 4(a-b): SEM of rice husk after addition of 3% NaOH, 500x and 1,000x magnification

Without addition of 3% NaOH the morphological form of rice husk was neater and regular, while after addition of 3% NaOH in Fig. 4, the morphological form of rice husk was damaged and irregular. Comparisons in Fig. 3 and 4 showed that pre-treatment using 3% NaOH effectively damages rice husk structure by destroying lignin structure in rice husk[23]. It can be explained that a strong bond between Na^+ and OH^- can break the ester bond between lignin, hemicellulose and cellulose[24]. Increasing the concentration of NaOH will loosen the fibre bond between lignin-hemicellulose so that the cellulose contained in rice husk is more easily processed[25]. Lignin binding of lignin fibres-hemicellulose will facilitate anaerobic microbes to digest cellulose into biogas. Addition of NaOH also causes the destruction of the structure of a biomass so it can be converted into sugar compounds[26]. Structural changes on the substrate can increase porosity to increase biodegradability. Increased biodegradability can facilitate microorganisms in the process of biogas formation[27].

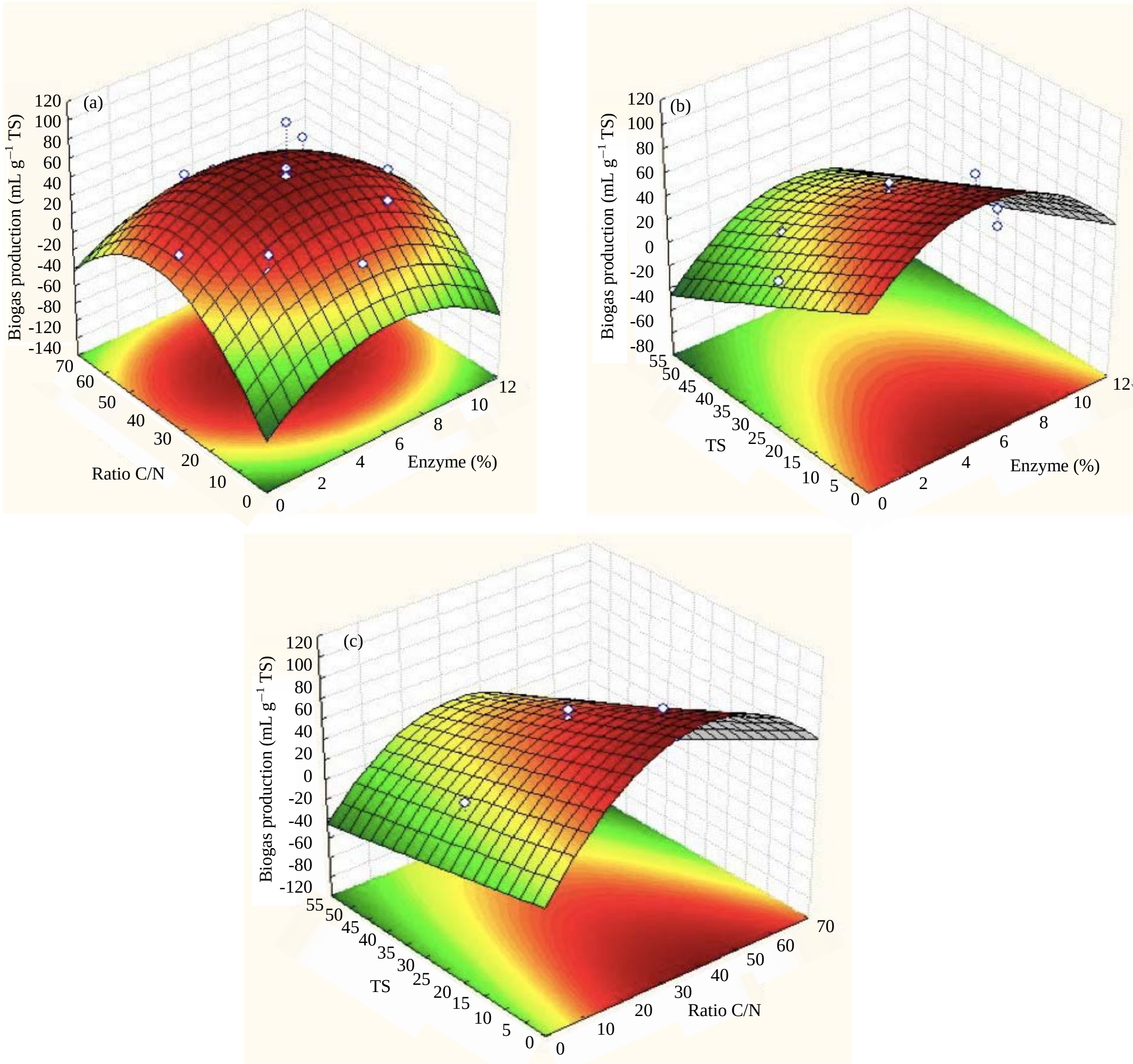

Fig. 5(a-c): Response surface of the effect of independent variables on biogas production, (a) C/N ratio and enzyme, (b) TS and enzyme and (c) TS and C/N ratio

Optimization of effect of enzyme, C/N ratios and total solid factor on biogas production: The response surface methodology was used to determine the optimum yield of biogas. The presentation of the result is shown by contour plot of Eq. 2. The maximum predicted biogas production was indicated by the surface confined in the smallest ellipse in the contour diagram[28].

The C/N ratio and enzyme were studied for the range of 20-50 and 3-9%, respectively. These ranges are encoded as -1 to +1 as shown in Table 1. Figure 5 showed the yield of biogas production as function of C/N ratio, total solid (TS) and Enzyme concentration. As the C/N ratio and enzyme concentration increase the biogas's yield also significantly increase until certain optimum condition. The optimum yield was achieved at 63 mL g^{-1} TS with C/N ratio of 38 and enzyme concentration of 6.6%. The increase of C/N ratio after optimum condition due to the decrease of nitrogen in the biodigester. The variation of the C/N values can affect the pH of a slurry. The increase in C/N ratio will give rise to more carbon dioxide (CO_2) formation which lead to lower pH value and the decrease of C/N ratio will enhance production of ammonia gas that could increase the pH which also lead to the detriment of the micro-organisms.

The interaction between enzyme concentration and total solid (TS) in biogas production is shown by Fig. 5b. The total solid shows the positive correlation to biogas production while by increasing the enzyme concentration will also increase the biogas yield until the optimum value was

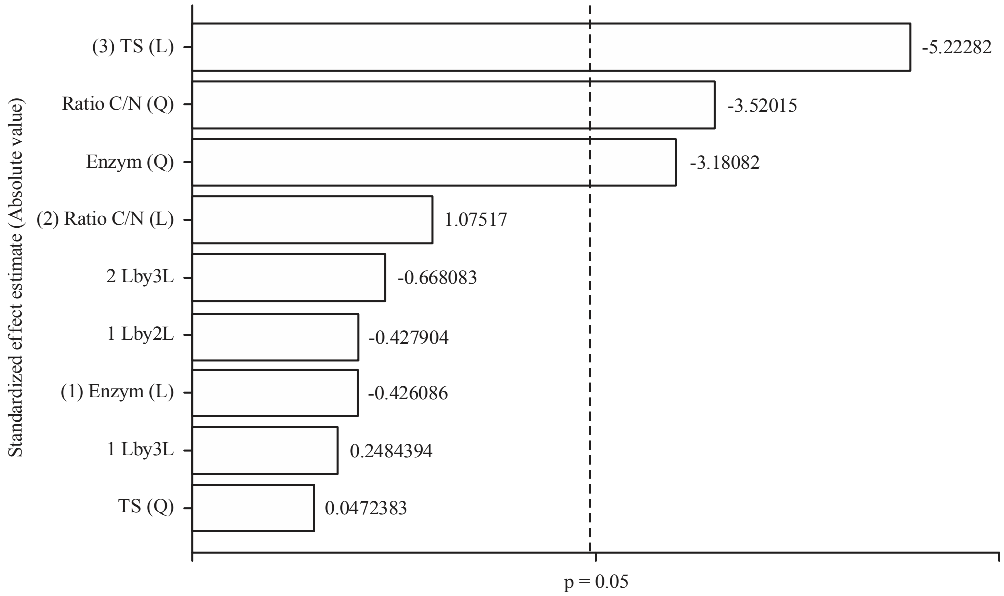

Fig. 6: Significance of the effect of all parameters on biogas production

achieved (63 mL g^{-1} TS). After concentration of enzyme exceed 6% the yield decreases due to its activity is slower at high pH. This high pH was caused by higher C/N ratio (Fig. 5a).

Finally, the significance of the effect of all parameters enzyme, C/N ratio and TS content on biogas production was presented by Pareto Chart in Fig. 6.

Figure 6 showed that at the 99.5% significance level, enzymes, C/N ratios and TS have a significant effect on biogas production ($p<0.05$). The influence of TS factor on biogas production is linear and on the C/N ratio and enzyme are quadratic. Figure 6 also showed that with a significance level of 99.5%, the linear C/N ratio and enzyme and the quadratic TS do not have a real effect and no significant interaction effects occur between the two factors or three factors ($p>0.05$). In addition, Fig. 6 showed that among these factors, linear TS factor is the most influential factor on biogas production.

The optimum values of each factor and the maximum biogas production were expressed quantitatively using the analytical method. The influence of several enzyme (X_1), C/N ratios (X_2) and TS (X_3) factors on biogas production (P) can be expressed by Eq. 2. The optimum condition of C/N ratio in the range 32-45 corresponds to the statement that the optimum C/N ratio for methane gas production is 20-35[29]. Then the results of the study, show that the optimum C/N ratio lies in the range of 20-30[30], with organic feedstock. Other studies were also carried out with variation of C/N ratio 20-35 and found that the optimum C/N ratio of 35 using rice husk raw material at concentration of TS 21% (SSAD)[6].

CONCLUSION

Chemical pre-treatment by using 3% NaOH could increase the yield of biogas production up to 4.5 times higher than without pretreatment. The productivity of biogas obtained from the pretreated substrate was 0.97 mL g^{-1} TS, which was almost 5 times higher than the substrate without pretreatment. The response surface optimization resulted the maximum biogas production of 64 mL g^{-1} TS which was obtained at enzyme concentration of 6%, C/N ratio of 35 and total solid of 27%.

The implications of this study derived from our finding on determining the optimum utilization of rice husk as alternative source for biogas production by solid state anaerobic digestion method. The searching optimum yield in this study can also be applied to other agricultural waste which have high cellulose contents. However, the study was also limited at the certain range of variables and need further study in evaluating other possibility variables such as the mixed substrates or variation of micro-organisms.

SIGNIFICANCE STATEMENT

The study discovers optimal operating conditions on biogas production from rice husk waste by enzyme, C/N ratio and TS factors so as to obtain high biogas productivity. Thus, a new theory of optimization of biogas production from rice husk waste can be obtained and useful when performing biogas production operations on a larger scale such as pilot scale and industrial scale.

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