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Response Surface Methodology to Optimize Methane Production from Mesophilic Anaerobic Co-Digestion of Oily-Biological Sludge and Sugarcane Bagasse

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Abstract: Oily-biological sludge (OBS) generated from petroleum refineries has high toxicity. Therefore, it needs an appropriate disposal method to reduce the negative impacts on the environment. The anaerobic co-digestion process is an effective method that manages and converts organic waste to energy. For effective anaerobic digestion, a co-substrate would be required to provide a suitable environment for anaerobic bacteria. In oily-biological sludge, the carbon/nitrogen (C/N) ratio and volatile solids (VS) content are very low. Therefore, it needs to be digested with organic waste that has a high C/N ratio and high VS content. This study investigates the use of sugarcane bagasse (SB) as an effective co-substrate due to its high C/N ratio and high VS content to improve the anaerobic co-digestion process with oily-biological sludge. The sugarcane bagasse also helps to delay the toxicity effect of the methane bacteria. Batch anaerobic co-digestion of oily-biological sludge was conducted with sugarcane bagasse as a co-substrate in twelve reactors with two-liter capacity, each under mesophilic conditions. The interaction effect of a C/N ratio of 20-30 and a VS co-substrate/VS inoculum ratio of 0.06-0.18 on the methane yield (mL CH_4/g $VS_{removed}$) was investigated. Before the anaerobic digestion, thermochemical pre-treatment of the inoculum and co-substrate was conducted using sodium hydroxide to balance their acidic nature and provide a suitable pH environment for methane bacteria. Design and optimization for the mixing ratios were carried out by central composite design-response surface methodology (CCD-RSM). The highest predicted methane yield was found to be 63.52 mL CH_4/g $VS_{removed}$, under optimum conditions (C/N ratio of 30 and co-substrate/inoculum ratio of 0.18).

Keywords: anaerobic co-digestion; oily-biological sludge; sugarcane bagasse; biogas; biomethane; bio-fuels; CCD-RSM

1. Introduction

Presently, among the greatest challenges and global problems for humanity in the current century are environmental pollution and energy insecurity [1]. Global warming, which is related to greenhouse gas emissions (especially CO_2) and depletion of fossil fuels during the current century, drives the push for alternative sustainable resources for energy [2]. Biomass is considered the best option for renewable and suitable energy that can replace a large portion of fossil fuels, besides solar and wind energy. Meanwhile, bioenergy will contribute to enhance developed countries' standard of life, as it is a potent source for biofuels [3–5].

Pollution control concurrent with bioenergy production can be achieved through different conversion processes for biomass to bioenergy. One of the most effective technologies to convert biomass to biofuel is anaerobic digestion [4]. Anaerobic digestion depends on different types of microbes, organic materials' characteristics, and different operational factors such as carbon/nitrogen (C/N) ratio, temperature (mesophilic and thermophilic), organic matter, pH, duration and so on [6–11]. Therefore, all factors affecting the anaerobic digestion process need to be considered for achieving cost-effective biogas yield as the main product from the anaerobic digestion process.

Subsequently, to meet the mentioned factors that affect the stability and effectiveness of the anaerobic digestion process, co-digestion with another substrate in many cases is required rather than mono-digestion to obtain the main goal, which is improving and enhancing biogas generation and quality [12]. For instance, some bioresources have a low C/N ratio and some have a high C/N ratio; the requirements for the anaerobic digestion process are that the C/N ratio should be between 20 and 30 and the pH between 6.2 and 8 [13,14]. Besides the main advantages of anaerobic co-digestion compared to mono-digestion such as balancing C/N ratio and pH, reduction of toxicity of some bioresources can be achieved through adulation with another nontoxic organic substrate to improve the kinetics of methane production [15,16]. Therefore, anaerobic co-digestion is necessary to obtain a balance among the factors that affect the anaerobic process for effective biogas production.

Researchers found that prior to the conversion process of the organic waste to biofuels, a pre-treatment process must be carried out to enhance the conversion process and final product. There are many pre-treatment methods for organic waste, and the selection of the suitable method mainly depends on the characteristics of the organic material and the type of final biofuel product. Pre-treatment for lignocellulosic biomass such as sugarcane bagasse will improve the hydrolysis process of the biomass due to the accessibility enhancement towards the rich fraction of cellulose. Therefore, the biomass becomes more accessible by bacteria and enzymes to convert valuable sugar in the biomass to the final products such as biogas and bioethanol [17–19]. As the anaerobic co-digestion process is a key factor of energy recovery in biogas form, a pre-treatment process is a necessary stage to enhance the digestion process, especially at the hydrolysis stage due to limitations that appear through this step [20]. Subsequently, pre-treatment to enhance and accelerate the hydrolysis stage must be implemented before the anaerobic co-digestion process, and the selected pre-treatment method depends on many factors such as the type of co-organic waste digested with the sludge.

Oily-biological sludge generated from wastewater treatment plants in petroleum refineries can be a source of substrate for second-generation biofuels [21,22]. Therefore, it can be digested anaerobically for biogas production. However, due to its lower C/N ratio and VS content, organic co-substrate, which has higher C/N and VS content, is needed to reach an adequate co-digestion process. Sugarcane bagasse can be a suitable waste material for an anaerobic co-digestion process to balance the C/N ratio and reduce the toxicity of oily-biological sludge to enhance biogas production. However, anaerobic co-digestion is a complex process; improper selection of the co-substrate and operational conditions can cause process instability or lower methane quality. Therefore, process modeling is necessary to control the operational parameters for better methane yield quality as well as to support lab-scale design pilot studies in this area. There are many mathematical models for designing anaerobic digestion and co-digestion processes; among them, there are statistical models that focus on the characterization of fundamental aspects of anaerobic co-digestion processes such as the relationship between input parameters (co-substrate/inoculum ratio, C/N ratio and temperature) and the design outputs such as methane quality and volatile solids reduction [23]. There are two main statistical models used for anaerobic co-digestion process design; they are central composite design which was used in this work (CCD) and simplex-centroid mixture design; both can be used for optimizing input parameters for better methane yield production [24].

Therefore, the objective of this study is to evaluate the feasibility of the anaerobic co-digestion process for oily-biological sludge (inoculum and substrate) with sugarcane bagasse (co-substrate) for biogas production. Another objective is to analyze the effect of batch design operational parameters (co-substrate/inoculum ratio and C/N ratio) under the mesophilic condition on the biomethane produced. We aim to optimize the process condition for the parameters through central composite design-response surface methodology (CCD-RSM).

2. Materials and Methods

2.1. Materials

The oily-biological sludge used was waste sludge from a petroleum refinery wastewater treatment plant (WWTP). The WWTP is an extended aeration activated sludge system. Oily-biological sludge was stored in a cold room at a temperature of ≤4 °C for less than one day to keep the original state of the material for its further characterization and pre-treatment process before co-digestion with sugarcane bagasse. Sugarcane bagasse was collected from Seri Iskandar, Malaysia. The bagasse was first manually cut to an average size of 15 cm and washed using tap water to remove trapped impurities and ligneous materials. The sugar cane bagasse was then dried in an oven at 105 °C for 24 hours to obtain a constant dry weight. The dried bagasse was then ground and milled to a size of 0.5 mm using a mechanical shredder and mill machines. Samples were subsequently stored in the cooling room at a temperature of ≤4°C for further characterization and pre-treatment process. Figure 1 shows the oily-biological sludge and sugarcane bagasse prior to the thermochemical pre-treatment and co-digestion processes.

Figure 1. (**a**) Raw oily-biological sludge; (**b**) sugarcane bagasse before pre-treatment; (**c**) sugarcane bagasse powder after mechanical pre-treatment and before thermochemical pre-treatment.

2.2. Pre-treatment of Oily-Biological Sludge and Sugarcane Bagasse

Oily-biological sludge was treated by a thermochemical pre-treatment method by using 1 g/L sodium hydroxide under 100 °C temperature, 150 rpm for 1 hour using a magnetic stirrer to enhance the digestibility of organic matter and to balance the pH of the batch mixtures during the anaerobic co-digestion process.

Sugarcane bagasse was treated using mechanical and thermochemical pre-treatment methods. The bagasse was shredded and milled to <0.5 mm particle size to enhance the surface area for easier accessibility by bacteria during the co-digestion process. Then, the bagasse powder was treated thermochemically by using sodium hydroxide for delignification to enhance the biogas production and balance the pH of the batch mixtures. Delignification was carried out under different dosages of NaOH and different times. NaOH at 1% and 2% (w/v) was added for a 1:10 solid:liquid ratio of sugarcane bagasse with distilled water for three periods, 45, 60 and 75 minutes respectively, under the temperature of 100 °C and 150 rpm using a magnetic stirrer.

The results of the pre-treatment process for sugarcane bagasse were tested using Chesson's method, and it was found that 1% NaOH, for 60 minutes, at 150 rpm, 100 °C and with a 1:10 solid liquid ratio was the best condition for the delignification process, and the lignin content was the minimum for the co-digestion process, at 13.50%.

2.3. Analytical Methods

Determination of total solids (TS), volatile solids (VS) and pH for oily-biological sludge was conducted according to American Public Health Association (APHA, 1998) standards. In the oily-biological sludge, carbon, hydrogen, nitrogen and sulfur (CHNS) were analyzed using a combustional elemental CHNS analyzer (Model: vario MICRO, Elemetar, Germany).

Proximate analyses for sugarcane bagasse to determine pH, ash moisture, fixed carbon and volatile matter were performed according to the Hach method and the ASTM D3172-89 method. Ultimate analyses to determine carbon, hydrogen, nitrogen and sulfur were measured with the same method used for the oily-biological sludge. Chemical composition analysis for bagasse to determine hemicellulose, cellulose and lignin was performed according to Chesson's method. Table 1 shows the main characteristics of treated oily-biological sludge (OBS) and treated sugarcane bagasse (SB).

Table 1. Main characteristics of treated oily-biological sludge and sugarcane bagasse.

Parameter	Unit	Oily-Biological Sludge	Unit	Dry Sugarcane Bagasse
Moisture Content	%	94.20	%	0
pH	N/A	8.70	N/A	7.21
TS	g/L	58.00	%	100
VS	g/L	50.46	%	87.80
C	% of TS	4.31	%	34.70
N	% of TS	0.30	%	0.26
C/N	N/A	14.42	N/A	132.69
Hemicellulose	N/A	N/A	%	10.25
Cellulose	N/A	N/A	%	62.05
Lignin	N/A	N/A	%	13.50

*N/A: Not applicable.

2.4. Experimental Procedures

In this study, the oily-biological sludge was digested under mesophilic anaerobic conditions with sugarcane bagasse as the co-substrate. Before the co-digestion process, the reactors were operated for two days for adaptation purposes. Twelve reactors with varying C/N and co-substrate/inoculum ratios were run under mesophilic anaerobic conditions. During the digestion process, biogas yield was measured daily using the water replacement method. The methane content in the biogas yield was sampled using a 3.5-liter sampling bag and analyzed using an Optima 7 biogas analyzer. After 32 days of digestion for each batch, the final volatile solids were measured. The final biomethane yield was calculated by measuring the cumulative biomethane yield per volatile solids removed. Then, the total biogas yield from each run was analyzed according to C/N and co-substrate/inoculum ratios and other parameters—temperature, pH and mixing. The optimized biomethane yield was evaluated in terms of maximum biomethane yield per volatile solids removed. The optimized ratio was validated in the lab through three replicants, and the experimental biomethane yield was compared with the predicted biomethane yield.

2.5. Experimental Design Through CCD-RSM

Central composite design with two level-two factors was selected to optimize the biomethane yield ($n = 2, \pm\,\alpha = 1.0$). The design consists of 12 runs: 4 factorial points, 4 axial points and 4 replicates' center points. The design was mainly based on the C/N ratio ranging from 20 to 30 [13], with concurrently different co-substrate/inoculum ratios. Table 2 shows the minimum and maximum values for the co-substrate and inoculum to design the mixing ratios through CCD-RSM.

Table 2. Levels of variables used for design optimization of methane production.

Independent Variable	Unit	Variable Level		
		−1 (Min)	0	1 (Max)
X_1 Sugarcane Bagasse	g	1	1.5	2
X_2 Oily-biological Sludge	g	193	243.5	294

The design of the minimum and maximum levels of the two factors sugarcane bagasse and oily-biological sludge was based on the C/N ratio to meet the requirements of a suitable environment for bacteria growth, which needs to be between 20 and 30. The following formula is used to calculate C/N ratio for composite materials:

$$R = \frac{Q_1(C_1 * (100 - M_1) + Q_2(C_2 * (100 - M_2)}{Q_1(N_1 * (100 - M_1) + Q_2(N_2 * (100 - M_2)} \tag{1}$$

where:

R	= C/N ratio;
Q_1, Q_2	= mass of materials "as is" or wet weight;
C_1, C_2	= carbon content of materials (%);
N_1, N_2	= nitrogen content of materials (%);
M_1, M_2	= moisture content of materials.

Experimental data given by CCD-RSM were used for generating the best fit for second-order polynomial regression in two variables as follows:

$$Y = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_{11}{X_1}^2 + \beta_{22}{X_2}^2 + \beta_{12}X_1X_2$$

where Y is the response of the dependent variable (mL CH_4/g $VS_{removed}$); β_0, β_1 and β_2 represent linear coefficients; β_{11} and β_{22} represent quadratic coefficients; β_{12} represents an interaction coefficient; X_1 and X_2 represent the independent variables, viz., carbon/nitrogen ratio (C/N) and volatile solids ratio (co-substrate/inoculum), respectively. Interactions between independent variables and their effective relationship with response were analyzed by performing ANOVA to check the model adequacy. The optimized parameters for the best methane production were investigated using two-dimensional and three-dimensional plotting, done on Design-Expert 10.0.

The minimum and maximum values for the factors were based on the C/N ratio. Table 3 shows the factors' mixing ratios given by the CCD-RSM; based on them, all C/N and co-substrate/inoculum ratios were calculated, and other operating parameters were set as needed.

Table 3. Experimental design of the model for maximum methane yield per volatile solids.

Run Order	Real Values		C/N Ratio	OBS VS Content (g)	SB VS Content (g)	Co-Substrate/Inoculum
	X_1	X_2				
1	1.5	243.5	24.2	100.1	10.8	0.11
2	1.5	243.5	24.2	100.1	10.8	0.11
3	1.0	243.5	21.1	100.1	7.2	0.07
4	1.5	294.0	22.6	100.1	9.0	0.09
5	1.0	294.0	20.0	100.1	6.0	0.06
6	2.0	294.0	25.1	100.1	11.9	0.12
7	1.0	193.0	22.8	100.1	9.1	0.09
8	2.0	193.0	30.0	100.1	18.2	0.18
9	1.5	243.5	24.2	100.1	10.8	0.11
10	1.5	243.5	24.2	100.1	10.8	0.11
11	2.0	243.5	27.1	100.1	14.4	0.14
12	1.5	193.0	26.5	100.1	13.5	0.13

* OBS VS = oily-biological sludge volatile solids (inoculum and substrate); SB VS = sugarcane bagasse volatile solids (co-substrate).

2.6. Setup of Experiment Operational Conditions

The anaerobic digester used to carry out the experiments is SOLTEQ TR37, as shown in Figure 2, which has 6 reactors. Each of the reactors has a volume of 2.5 L, with working volume of 2 L. The mixtures of factors as mentioned in Tables 2 and 3 were pre-treated and transferred to the digesters. The digesters were purged by nitrogen gas to ensure oxygen gas was removed to provide an anaerobic environment condition. The operating temperature was set and controlled at 37.0 ± 0.5 °C by heating sensors and the control panel positioned in the digester.

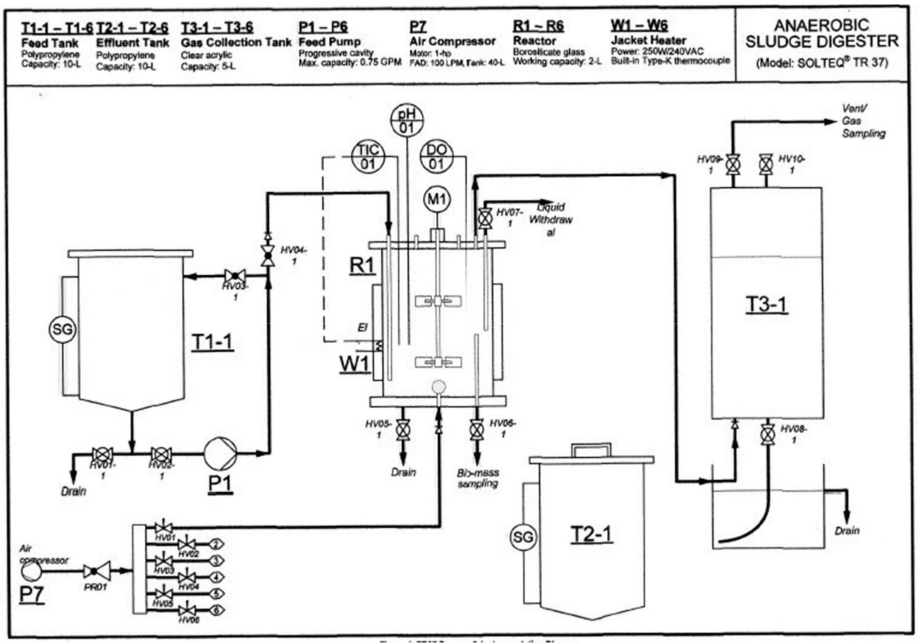

Figure 2. Sludge anaerobic digester diagram model: SOLTEQ TR37. R1: reactor; T1-1: feed tank; T2-1: effluent tank; T3-1: gas collection tank; W1: jacket heater.

The mixing speed for all reactors of the experiments was set at 60 rpm. All reactors have pH sensors. The pH of the mixtures for each digester was automatically recorded by the digester periodically every 1 hour. All the batch mixtures' durations were set at 32 days. The produced biogas was measured daily through a water displacement method and sampled every three days by sampling bags to measure the methane content by using an Optima 7 biogas analyzer.

3. Results and Discussion

Table 4 shows the experimental and predicted methane yield per g $VS_{removed}$ for the two variables through the designed experiments given by CCD-RSM. The methane yield was determined by calculating the cumulative methane yield throughout the duration (32 days) and then dividing by removed volatile solids per batch (2 liters).

Table 4. Design matrix for experimental vs predicted methane yield.

Run Order	Real Values		Volatile Solids Removed Per Batch (g)	Experimental Methane Yield (mL CH_4/g $VS_{removed}$)	Predicted Methane Yield (mL CH_4/g $VS_{removed}$)
	X_1	X_2			
1	1.5	243.5	34.8	36.1	36.9
2	1.5	243.5	35.1	37.5	36.9
3	1.0	243.5	32.5	31.5	30.3
4	1.5	294.0	33.2	31.7	32.1
5	1.0	294.0	32.3	30.3	31.1
6	2.0	294.0	39.2	41.2	40.0
7	1.0	193.0	33.4	31.9	32.3
8	2.0	193.0	46.2	65.1	63.5
9	1.5	243.5	36.1	38.6	36.9
10	1.5	243.5	35.6	37.0	36.9
11	2.0	243.5	42.2	47.6	50.3
12	1.5	193.0	39.8	43.3	44.4

3.1. Statistical Analysis of Co-Digestion Process Optimization through CCD-RSM

Experimental results for the co-digestion process were themed to the CCD-RSM to assess and analyze the effect of mixing variable ratios based on their C/N and co-substrate/inoculum ratios. The results were fitted to the second-order polynomial equation. Therefore, the model regression for the methane yield (mL CH4/g $VS_{removed}$) for the real values was given as follows:

$$\text{Methane yield (mL } CH_4 \text{ /g } VS_{removed}) = 19.667 + 32.296\ X_1 - 0.059\ X_2 + 13.810\ X_1^2 + 0.00054\ X_2^2 - 0.220\ X_1X_2 \quad (2)$$

Therefore, Equation (2) is the mathematical model resolved by the CCD to find out the predicted optimized methane yield based on the designed experimental methane yield for each independent variable during the anaerobic co-digestion process. ANOVA analysis was carried out by the CCD-RSM to check and assess the significant and mathematical model adequacy. Table 5 shows the ANOVA results of the design.

Table 5. ANOVA results for the response surface quadratic model.

Source	df	Sum of Squares	Mean Square	F-Value	p-Value Prob > F
Model	5	1007.75	201.56	62.19	<0.0001
X_1	1	603.61	603.61	186.26	<0.0001
X_2	1	229.03	229.03	70.67	0.0002
X_1X_2	1	123.88	123.88	38.23	0.0008
X_1^2	1	31.79	31.79	9.81	0.0203
X_2^2	1	5.21	5.21	1.61	0.2519
R^2		0.98			
Adj-R^2		0.97			
CV^p		4.58			
Std. Dev.		1.80			
Lack of Fit	3	16.26	5.42	5.11	0.1067
Pure Error	3	3.18	1.06		

* Probability value ($p < 0.05$ assumed significant, $p > 0.05$ assumed not significant); df = Degree of freedom.

Model F- and p-values were observed to be 62.19 and <0.0001, respectively, which indicates that the model has a significant contribution against the outputs at 95% confidence level ($p < 0.05$).

Meanwhile, a low F-value (5.11) and high p-value (0.1067), related to the lack of fit, indicated that the model is not significant relative to the pure error. A very low value of the coefficient of variance (4.58) indicated a high reliability of the experimental data as well as a high level of accuracy. Researchers have established that good statistical models of best fit should have an R^2 value between 0.75 and 1 [25,26]; in the present study, the R^2 was observed to be 0.98, which means that the model could explain 98% of the response variability. Linear model values (X_1, X_2), quadratic model value ($X_1{}^2$), and interactive model values (X_1X_2) were found to be significant, with p-values <0.05. Figure 3 (a and b) shows the normal probability plot of residuals and predicted vs actual methane yield. Plots show that there is no abnormality in the experimentation work, therefore the model is successful in predicting methane yield.

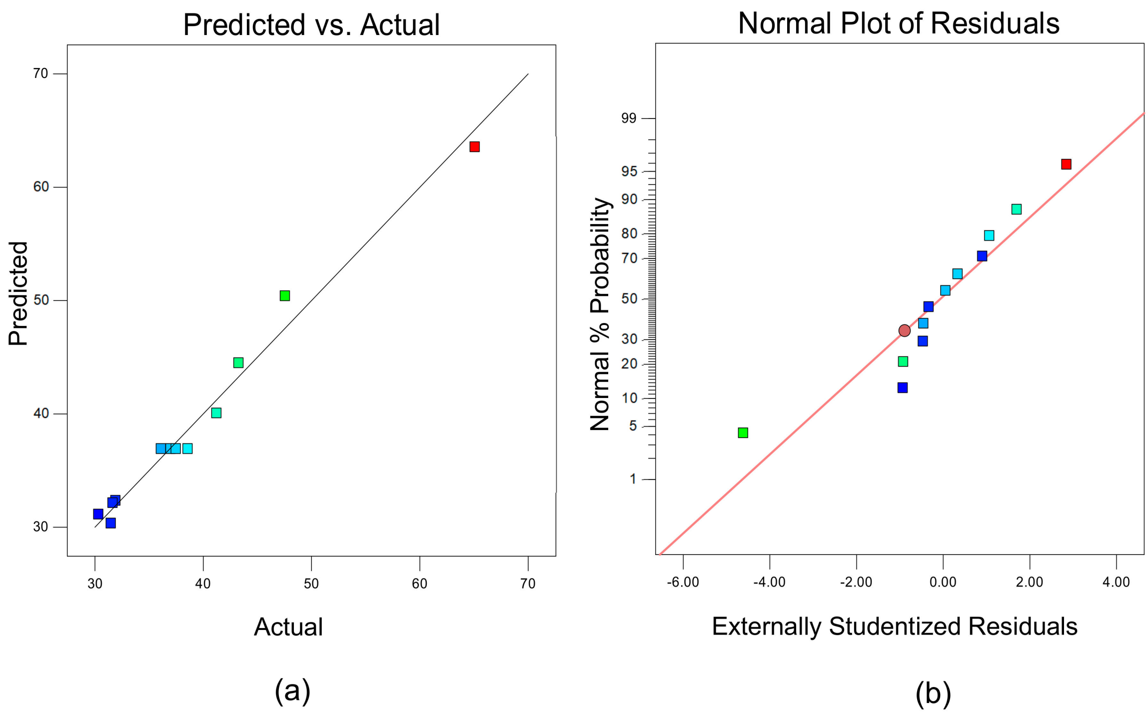

Figure 3. (**a**) Plot of residuals and normal probability; (**b**) plot of actual vs predicted values.

3.2. Interactive Effect of Process Variables' Ratios on Methane Yield

The independent variables' interactive effect on the methane yield was analyzed through ANOVA, contour plots and 3D surface plots. From Table 5, the statistical difference between C/N and co-substrate/inoculum ratios was found to be significant ($p < 0.05$). Based on Figure 4, there is a significant interaction effect between C/N and co-substrate/inoculum ratios on methane yield. Minimum C/N and co-substrate/inoculum ratios resulted in the minimum methane yield of 31.10 mL CH_4/g $VS_{removed}$, while maximum C/N and co-substrate/inoculum ratios resulted in the maximum methane yield of 63.52 mL CH_4/g $VS_{removed}$. The figures reveal that there is increasing methane yield with sugarcane bagasse increment. Once the sugarcane bagasse proportion increased the C/N ratio, co-substrate/inoculum ratio and biomethane yield increased as well, resulting in decreasing nitrogen content in the system and subsequently lower ammonia inhabitation [27,28]. Meanwhile, oily-biological sludge's toxicity is high; this results in a reluctant environment for bacterial activities [29]. Therefore, increasing sugarcane bagasse content will act as an adsorbate agent for toxic elements, and this will delay the toxicity effect on bacterial activity and lead to increased biomethane yield. A main factor affecting the process stability is the pH value; for the maximum biomethane yield, it is found to be between an initial pH of 7.6 and a final pH of 7.4, and the best range for an anaerobic co-digestion healthy environment for bacteria needs to be between 6.2 and 8.0 [13].

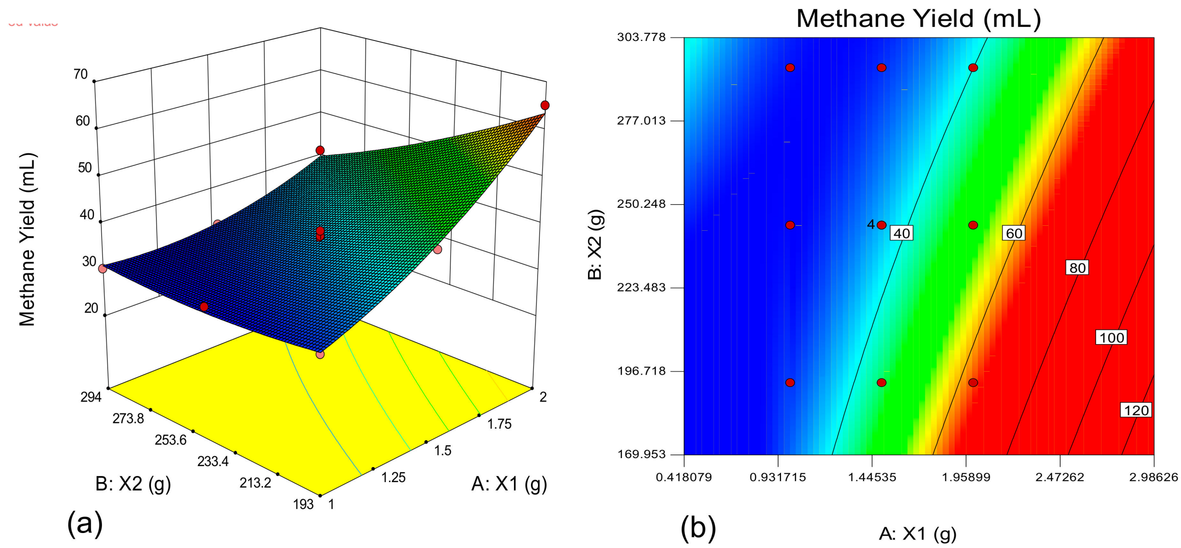

Figure 4. Interactive effect of C/N and co-substrate/inoculum ratios on methane yield: (**a**) 3D response surface; (**b**) contour plots.

3.3. Model Validation for Optimum Conditions

Generally, to check the validity of the suggested model for maximum methane yield, one needs to conduct lab experiments for the optimum conditions. Three experiments were conducted for C/N ratio 30, co-substrate/inoculum ratio 0.18, 37 °C, 60 rpm and 32 days. The average methane yield obtained from the validation experiments was 64.85 mL CH_4/g $VS_{removed}$; that is very close to the predicted value of 63.52 mL CH_4/g $VS_{removed}$. Therefore, the suggested model for maximum methane yield for the co-digestion process is accepted.

4. Conclusions

From this study, it can be concluded that sugarcane bagasse is an effective co-substrate for the batch anaerobic co-digestion process with oily-biological sludge for methane yield. The validated maximum methane yield was found to be 64.85 mL CH_4/g $VS_{removed}$ at the optimal conditions of a C/N ratio and co-substrate/inoculum ratio of 30 and 0.18, respectively, while the predicted methane production was found to be 63.52 mL CH_4/g $VS_{removed.}$ Sugarcane bagasse balanced the inappropriately low C/N ratio for oily-biological sludge degradation and delayed the high-toxicity effect of oily-biological sludge to provide a suitable environment for methane bacteria. Pre-treatment of the inoculum and co-substrate using thermochemical pre-treatment resulted in balancing the acidic nature of the inoculum and co-substrate to provide suitable pH values between 6.2 and 8.0 to avoid process inhabitation by volatile fatty acids or ammonia accumulation. Also, to increase the digestibility of the organic matter. The proper response variable for the co-digestion process was methane yield (mL CH_4/g $VS_{removed}$).

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