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Sugarcane Bagasse as a Co-Substrate with Oil-Refinery Biological Sludge for Biogas Production Using Batch Mesophilic Anaerobic Co-Digestion Technology: Effect of Carbon/Nitrogen Ratio

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Abstract: Man-made organic waste leads to the rapid proliferation of pollution around the globe. Effective bio-waste management can help to reduce the adverse effects of organic waste while contributing to the circular economy at the same time. The toxic oily-biological sludge generated from oil refineries' wastewater treatment plants is a potential source for biogas energy recovery via anaerobic digestion. However, the oily-biological sludge's carbon/nitrogen (C/N) ratio is lower than the ideal 20–30 ratio required by anaerobic digestion technology for biogas production. Sugarcane bagasse can be digested as a high C/N co-substrate while the oily-biological sludge acts as a substrate and inoculum to improve biogas production. In this study, the best C/N with co-substrate volatile solids (VS)/inoculum VS ratios for the co-digestion process of mixtures were determined empirically through batch experiments at temperatures of 35–37 °C, pH (6–8) and 60 rpm mixing. The raw materials were pre-treated mechanically and thermo-chemically to further enhance the digestibility. The best condition for the sugarcane bagasse delignification process was 1% (*w/v*) sodium hydroxide, 1:10 solid-liquid ratio, at 100 °C, and 150 rpm for 1 h. The results from a 33-day batch anaerobic digestion experiment indicate that the production of biogas and methane yield were concurrent with the increasing C/N and co-substrate VS/inoculum VS ratios. The total biogas yields from C/N 20.0 with co-substrate VS/inoculum VS 0.06 and C/N 30.0 with co-substrate VS/inoculum VS 0.18 ratios were 2777.0 and 9268.0 mL, respectively, including a methane yield of 980.0 and 3009.3 mL, respectively. The biogas and methane yield from C/N 30.0 were higher than the biogas and methane yields from C/N 20.0 by 70.04 and 67.44%, respectively. The highest biogas and methane yields corresponded with the highest C/N with co-substrate VS/inoculum VS ratios (30.0 and 0.18), being 200.6 mL/g VS_{removed} and 65.1 mL CH₄/g VS_{removed}, respectively.

Keywords: anaerobic co-digestion; oily-biological sludge; sugarcane bagasse; biogas; hazard waste management; biomethane

1. Introduction

Environmental pollution due to rapid waste generation and energy insecurity has become a global challenge in recent years. The combustion of fossil fuels from five sectors, namely energy generation, food and agriculture, transport, industry and economy, and

households, is considered the primary source of environmental pollution and greenhouse gas emissions [1]. Although the eventual depletion of fossil fuels will significantly reduce greenhouse gas emissions, it will also result in energy insecurity. Therefore, resolving the issues of environmental pollution and energy insecurity gradually through carbon recovery from non-sustainable and sustainable resources is a global concern and of interest for researchers [1–4].

Organic waste generated from industrial activities, such as biological industrial sludge, is toxic because it contains heavy metals and has adverse impacts on humans, the air, soil, and water. In addition to this, it is estimated that globally, around one billion tonnes of agricultural waste are generated annually, and 80% of this waste is organic [5,6]. This organic waste is composed of biodegradable and non-biodegradable portions. The biodegradable portion can be converted into water, methane, and carbon dioxide by microorganisms during aerobic and anaerobic digestion processes as an effective technology for high moisture content sludge compared to other current technologies for energy recovery from the sludge, such as combustion, pyrolysis, and gasification [7–10]. Therefore, non-sustainable organic waste generated from industrial activities, such as biological sludge generated from industrial wastewater effluent treatment systems, can contribute together with sustainable organic waste generated from biomass to energy recovery to reduce environmental pollution and contribute to a gradual transition from non-renewable to renewable energy.

Energy production using anaerobic digestion technology for high-strength waste treatment which is not residential in nature has been applied historically, with proven and valuable outcomes [11,12]. Due to the diverse characteristics related to proximate and ultimate analysis of the organic waste and the need for a suitable microorganism environment, such as nutrient balance, pH temperature and so on, anaerobic co-digestion is an effective way to enhance the digestion process for better biogas quantity and quality [13–15]. The anaerobic co-digestion process can reduce environmental pollution and improve the circular economy through the conversion of low value materials such as organic waste, either agricultural, residential or industrial, to high value materials such as biofuels [16–18].

During the treatment of wastewater in petroleum oil refineries, proper management of toxic organic oily-biological sludge is needed. The anaerobic digestion process is an efficient method to treat organic waste by converting most of the organic matter into biogas. Thus, oily-biological sludge can be digested to produce biogas and reduce its negative impacts on the environment. However, the carbon/nitrogen (C/N) ratio for oily-biological sludge is low and does not meet the 20–30 ratio requirements of the anaerobic digestion environment [19]. Hence, anaerobic co-digestion with another organic waste that has a high C/N ratio is needed. Sugarcane bagasse is a lignocellulosic organic waste that can be treated bio-chemically using anaerobic digestion technology for biogas production [20]. Meanwhile, it has a high C/N ratio and is readily available, as 54 million dry tonnes of it are generated annually around the world [21]. Therefore, sugarcane bagasse can be digested as a co-substrate with oily-biological sludge which acts as a substrate and inoculum for biogas production. The objectives of this study were to investigate the effect of C/N ratios together with co-substrate volatile solids (VS)/inoculum VS ratios during the anaerobic co-digestion process for pre-treated oily-biological sludge and sugarcane bagasse. To increase the C/N ratio, we need to increase the dosage of sugarcane bagasse. Subsequently, the co-substrate VS/inoculum VS ratio will increase. Therefore, the effect on biogas and methane yield is related at the same time to C/N and co-substrate VS/inoculum VS ratios. The experiments were carried out under mesophilic conditions, and the raw materials were mechanically and thermo-chemically pre-treated to improve the anaerobic co-digestion process and enhance biogas production.

2. Materials and Methods

2.1. Materials

Sugarcane bagasse was collected from a night market at Seri Iskandar, Malaysia. The bagasse was first manually cut to an average of 15 cm and then cleaned using tap water

to remove any trapped impurities and ligneous. The bagasse was then dried in an oven at 105 °C for 24 h to obtain a consistent dry weight. Next, the dried bagasse was ground and milled to smaller than 0.5 mm using a mechanical shredder machine (Granulator WSGP-230) and miller machine (Puluerisette 16). The grinding and milling of the dried bagasse increased its surface area and digestibility by the bacteria during the anaerobic co-digestion process [22,23].

The dried sugarcane bagasse was treated thermo-chemically using sodium hydroxide in different ratios, temperatures, and periods to reduce lignin content as much as possible, which was recalcitrant to the hydrolysis process [24]. Due to the acidic nature of the sugarcane bagasse, mechanical and alkali-thermal pre-treatment methods were selected as effective methods to enhance the delignification and degradation process for better biogas yield and to increase the pH to meet the required pH range for an anaerobic digestion process, between 6 and 8 [19,25–27]. The thermo-chemical pre-treatment ratios were 1% and 2% (*w/v*) NaOH for 45, 60, and 75 min. The solid/liquid ratio was 1:10, the temperature was 100 °C, and the rpm was 150 rpm.

The oily-biological sludge is a waste-activated sludge that was collected from a petroleum oil refinery wastewater effluent treatment system and stored in a cooling room below 4 °C for less than 24 h before proceeding with the co-digestion process. Pre-treatment for the sludge was needed to enhance the degradation of the organic matter [28]. Due to the acidic nature of the oily-biological sludge, the alkali-thermal pre-treatment method was selected as an effective method to enhance the degradation process for better biogas yield and to increase the pH to meet the required pH range for the anaerobic digestion process, between 6 and 8 [19,29,30]. The sludge was thermo-chemically pre-treated using sodium hydroxide 1 g/L, at 100 °C, 150 rpm for 1 h to increase its pH as it has an acidic nature to comply with the required range of pH during anaerobic digestion process, between 6–8.

2.2. Analytical Methods, Design and Operational Setup

Proximate analysis for the treated oily-biological sludge (TOBS) was conducted according to the American Public Health Association (APHA), 1998 methods to determine the total solids (TS) and volatile solids (VS). The ultimate analysis was conducted to determine the carbon, hydrogen, nitrogen, and sulfur (CHNS) elements using the CHNS elemental machine (Vario MICRO) to design the mixing ratios according to the C/N ratio for the batch experiments.

The proximate analysis for the treated sugarcane bagasse was performed in accordance with the American Society for Testing and Materials (ASTM) D3172-89 standard. The pH of the sugarcane bagasse was determined according to Hach method. The ultimate analysis was performed according to ASTM D5373 standard using the same elemental machine to determine the C/N ratio for the mixtures design. The chemical composition to determine hemicellulose, cellulose and lignin was measured according to Chesson's method to compare and analyse the effect of thermo-chemical pre-treatment method on lignin content.

The functional groups present in the raw and treated sugarcane bagasse were determined by Fourier transform infrared (FTIR) analysis. FTIR analysis was conducted to investigate the effects of thermo-chemical pre-treatment on the functional groups, namely hemicellulose, cellulose, and lignin, using a PerkinElmer analyzer.

Supra 55 VP (Carl-Zeiss AG, Oberkochen, Germany) was used to analyse the surface morphology of raw sugarcane bagasse (RSCB), treated sugarcane bagasse (TSCB), raw oily-biological sludge (ROBS), and treated oily-biological sludge (TOBS) using a variable pressure scanning electron microscope. The images were taken for both materials before and after the thermo-chemical pre-treatment at different magnifications to observe and analyse the effects of the pre-treatment method. The design of mixtures was conducted

according to the following equation [31], in order to achieve mixtures for both materials between 20 and 30.

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

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.

The SOLTEQ TR37 sludge anaerobic digester was used to carry out this study [32]. The size of the digester used for this study was 2.5 L, with a working volume of 2 L. The digester’s temperature was set manually using the digester control panel at $35.0\text{--}37.0 \pm 0.5$ °C, with a mixing speed of 60 rpm for 33 days. The produced biogas was measured every day using a water displacement method, and the content was measured every three days using the German Optima 7 biogas analyzer. The one-way ANOVA test using Microsoft Excel 2010 was used to compare the biogas and methane yield from different reactor groups based on the C/N ratio and co-substrate VS/inoculum VS increments. The central point C/N ratio mixture was repeated four times to verify the experiment’s design. In addition, validation for the maximum biogas and methane yield was carried out using 3 replications for the best ratios.

3. Results and Discussion

3.1. Pre-Treatment Process Analysis, Characterization and Experimental Design

3.1.1. Thermo-Chemical Pre-Treatment of Raw Sugarcane Bagasse (RSCB)

After mechanical pre-treatment of RSCB was carried out to below a 0.5 mm particle size, thermo-chemical pre-treatment was conducted using sodium hydroxide to minimize the lignin content and enhance biogas production. Sodium hydroxide was used in different ratios and periods. The hemicellulose, cellulose, and lignin contents before and after the pre-treatment are tabulated in Table 1. The results show that 1% sodium hydroxide at 100 °C and 150 rpm for 1 h was the best combination pre-treatment to minimize the lignin content from sugarcane bagasse. The lignin content decreased to 13.50%, while the cellulose content increased to 62.05%. This will enhance the hydrolysis process and provide a good source of a digestible, accessible fraction for the microorganisms, which is the cellulose portion [24,25]. Three replicates for the best pre-treatment ratio were conducted and the average hemicellulose, cellulose, and lignin contents were 10.55, 62.45 and 13.60%, respectively, with the significance level being more than 95%. Therefore, this ratio was selected for the ultimate and proximate analysis of TSCB, and consequently for the batch design mixtures. Scanning electron microscopy (SEM) and FTIR were used to show the effect of the pre-treatment through surface morphology and functional group changes.

Table 1. Results of delignification process for raw sugarcane bagasse (RSCB) at a temperature of 100 °C and stirring speed of 150 rpm.

	Hemicellulose (%)	Cellulose (%)	Lignin (%)
RSCB	24.32	36.20	28.03
TSCB 1%, 45 min	8.03	50.40	25.30
TSCB 1%, 60 min	10.25	62.05	13.50
TSCB 1%, 75 min	8.17	58.17	16.43
TSCB 2%, 45 min	8.37	56.80	17.87
TSCB 2%, 60 min	7.90	51.70	23.40
TSCB 2%, 75 min	4.95	64.35	14.45

3.1.2. Proximate and Ultimate Analysis for Treated Oily-Biological Sludge (TOBS) and Treated Sugarcane Bagasse (TSCB)

According to Table 2, the pH for TOBS and TSCB has a base nature induced by chemical pre-treatment using NaOH. This will provide a pH of 6.2 to 8, as recommended for an effective anaerobic co-digestion process; during anaerobic co-digestion, the acetogenesis phase has an acidic nature, and that will balance the base nature of the mixtures to be within the recommend pH range [19,33]. The VS for both materials comprise a high portion of the total solids, and this is a good indicator for high biogas yield, as most of the volatile matter will be converted to gas.

Table 2. The main ultimate and proximate analysis of treated oily-biological sludge (TOBS) and treated dry sugarcane bagasse.

Parameter	Unit	TOBS	Unit	TSCB
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

From Table 2, the C/N ratio for TOBS and TSCB was found to be 14.42 and 132.692, respectively, at base. The high C/N ratio for TSCB will balance the low C/N ratio of TOBS to provide a proper environment for microorganisms, with a C/N ratio between 20 and 30. These analyses were used in the study of methane modelling through surface response methodology for the anaerobic co-digestion of both materials [32,34].

3.1.3. FTIR Analysis for Raw Sugarcane Bagasse (RSCB) and Treated Sugarcane Bagasse (TSCB)

This analysis was conducted to determine the functional groups of RSCB and TSCB to analyse the changes induced by thermo-chemical pre-treatment. Figure 1a,b show the FTIR spectra for RSCB and TSCB, respectively. The variation bonds given by the FTIR spectra for the RSCB are shown in Table 3. The variation bonds mainly corresponded to cellulose, hemicellulose, and lignin. Figure 1a shows a strong peak at 3405.76 cm^{-1} ; this indicates the presence of the O-H stretching and NH group. The OH is related to polyphenol and alcohol, which was present in cellulose and lignin [35]. The peak at 2918.97 cm^{-1} reflects the C-H stretching, indicating the presence of methylene in the cellulose and hemicellulose [36,37]. The peak at 1733.42 cm^{-1} reflects the C=O stretching of acetal groups available in hemicellulose [38]. The peak at 1633.70 cm^{-1} reflects the C=O stretching group carbonyl of ketonic groups found in lignin [37]. The peaks at 1605.30 and 1513.89 cm^{-1} represent the aromatic skeleton C=C available in lignin [39–41]. The peaks at 1427.82 and 1375.33 cm^{-1} signify the CH₂ asymmetric stretching and C-H bend available in lignin and cellulose, respectively [42,43]. The peaks at 1249.50 and 1162.91 cm^{-1} describe the C-O stretching and C-O-C asymmetrical stretching available in hemicellulose and cellulose, respectively [38,40]. Finally, the peak at 1052 cm^{-1} may be due to the C-O stretching group available in cellulose [44].

The variation bonds given by the FTIR spectra for the TSCB are tabulated in Table 4. The strong peak at 3432.35 cm^{-1} , as shown in Figure 1b, indicates that the O-H stretching and NH group, and the OH is related to the polyphenol and alcohol present in the cellulose and lignin [35]. The peak at 2912.80 cm^{-1} reflects the C-H stretching found in methylene, which is present in the cellulose and hemicellulose [36,37]. The peak at 1600.03 cm^{-1} reflects the C-H skeletal variation available in lignin [34]. The peak at 1421.81 cm^{-1} presents the C-H bending present in cellulose [45]. Finally, the peaks at 1163.45 and 1057.39 cm^{-1}

indicate the C-O-C asymmetrical stretching and C-O stretching available in cellulose and hemicellulose [38,44,46]. The FTIR spectra analysis for RSCB and TSCB proved that the thermo-chemical pre-treatment for the RSCB induced a positive effect in the chemical structure, where TSCB had a lower lignin content compared to RSCB. Therefore, the thermo-chemical pre-treatment for the RSCB was successful in reducing the lignin content, which inhibited the anaerobic co-digestion process. The TSCB also enhanced the biogas production as the major fraction contents were cellulose and hemicellulose, which were the primary sources for the microorganism activities during the anaerobic co-digestion process with oily-biological sludge.

Figure 1. (a) FTIR spectrum of RSCB (b) FTIR spectrum of treated sugarcane bagasse (TSCB).

Table 3. FTIR characteristics of RSCB.

Bagasse	Frequency (cm ⁻¹)	Possible Assignment
RSCB	3405.76	O-H Stretching and NH group (cellulose and lignin)
	2918.97	C-H Stretching (cellulose and hemicellulose)
	1733.42	C=O stretching (Hemicellulose)
	1633.70	C=O stretching (lignin)
	1605.30	Aromatic skeleton C=C (lignin)
	1513.89	Aromatic skeleton C=C (lignin)
	1427.82	CH ₂ asymmetric stretching (lignin)
	1375.33	C-H bend (cellulose)
	1329.38	C-F stretch
	1249.50	C-O stretching (hemicellulose)
	1162.91	C-O-C Asymmetrical stretching (cellulose and hemicellulose)
	1052.00	C-O stretching (cellulose)
	833.09	C-H bending
	606.81	C-Br stretch

Table 4. FTIR characteristics of TSCB.

Bagasse	Frequency (cm ⁻¹)	Possible Assignment
TSCB	3432.35	O-H Stretching and NH (cellulose and lignin)
	2912.80	C-H Stretching (cellulose and hemicellulose)
	1600.03	Aromatic skeletal C=C (lignin)
	1421.81	C-H bending (cellulose)
	1163.45	C-O-C asymmetrical stretching (cellulose and hemicellulose)
	1057.39	C-O stretching (cellulose)
	657.78	≡C-H bend

3.1.4. Surface Morphology of Raw Sugarcane Bagasse (RSCB) and Treated Sugarcane Bagasse (TSCB)

The SEM observations for the RSCB and TSCB depicted in Figures 2 and 3 show an apparent physical change in the inner and outer surface induced by the thermo-chemical pre-treatment method. Figure 2a shows the general view for RSCB. There are two structures indicated by arrows S and P.

The arrow S indicates the fiber structure for the outer surface of the RSCB, which is usually the lignin portion. In contrast, the arrow P indicates the pith structure of the RSCB, which is the cellulose and hemicellulose fraction. Figure 2b shows that the TSCB also had two structures, indicated by the arrow S for fiber and P for pith. Based on Figure 2a,b, it was evident that RSCB had a higher fiber structure portion compared to TSCB, which indicated that the thermo-chemical pre-treatment method had a significant effect in reducing the lignin content.

The fiber structure of RSCB was more structured and attached compared to TSCB, which was independent and partially unattached. This indicates that the thermo-chemical pre-treatments broken down the bonds of lignin content at the outer surface, and reduced the inhabitation effect of the low unstructured lignin content during the anaerobic co-digestion process. Apart from that, it also increased the accessibility to the cellulose fraction during the hydrolysis process by bacteria [47].

Figure 3c,d show the magnification of pith structures for RSCB and TSCB. It is apparent that the pith structure of TSCB was more fragile and fragmented compared to the pith structure of RSCB. This indicates that the thermal pre-treatment also affected the pith structure, and a portion of the hemicellulose fraction was removed during the pre-treatment process which led to small pores, as shown in Figure 3d.

Figure 2. (a) General view of the RSCB sample shows the fibers (outer surface) and pith (inner surface), (arrows S and P). (b) General view of the TSCB sample shows the fibers (outer surface) and pith (inner surface), (arrows S and P).

Figure 3. (a) Magnification of fiber structure for RSCB. (b) Magnification of pith structure for TSCB. (c) Magnification of pith structure for RSCB. (d) Magnification of pith structure for TSCB.

3.1.5. Scanning Electron Microscopy of ROBS and TOBS

The SEM observations for the ROBS and TOBS, as illustrated in Figures 4 and 5, show a clear physical change in the flocs surface induced by thermo-chemical pre-treatment method. Figure 4 shows the general view for ROBS and TOBS, whereas Figure 5 shows the magnification of flocs surfaces for ROBS and TOBS. The alkaline-thermo pre-treatment induced a physical effect in the surface flocs

Figure 4. (a) ROBS flocs morphology. (b) TOBS flocs morphology.

Figure 5. (a,c) Magnification of ROBS floc surface. (b,d) Magnification of TOBS floc surface.

Figure 5a,c show the magnifications of ROBS flocs, while Figure 5c,d show the magnification of the flocs surface for TOBS. The flocs surface of TOBS became more compact with smaller voids ratio compared to the flocs surface of the ROBS. Furthermore, the pre-treatment caused more swelling and a disrupted structure in the flocs of TOBS. Therefore, the faster decomposition of the flocs by the microorganism increased the production of methane yield during anaerobic co-digestion. Similar reports for different types of sludge were reported in the literature [48,49].

3.1.6. Mixing Ratios of Treated Oily-Biological Sludge (TOBS) and Treated Sugarcane Bagasse (TSCB) to Obtain C/N Ratios between 20 and 30

Twelve batch experiments, including four replicates for the central point (medium C/N ratio 24.2) to increase the significance and confidence levels, were designed using Equation (1), based on the ultimate and proximate results shown in Table 2, to obtain C/N ratios between 20 and 30. Each batch contained 2 L of TOBS and a matching portion of sugarcane bagasse to meet the C/N ratio, as shown in Table 5. The density of the TOBS was similar to water. There were three main responses in this study, namely, biogas yield, methane content, and VS removed. Prediction of the total methane yield has been published as a part of this project [32]. Table 5 shows the ratios of TOBS and TSCB added to the batch to obtain the desired C/N ratios.

Table 5. Quantities of TOBS and TSCB added to obtain carbon/nitrogen (C/N) ratios between 20 and 30.

Run No.	TSCB (g)	TOBS (g)	C/N	Co-Substrate VS/Inoculum VS
1	1.0	294.0	20.0	0.06
2	1.0	243.5	21.1	0.07
3	1.5	294.0	22.6	0.09
4	1.0	193.0	22.8	0.09
5	1.5	243.5	24.2	0.11
6	1.5	243.5	24.2	0.11
7	1.5	243.5	24.2	0.11
8	1.5	243.5	24.2	0.11
9	2.0	294.0	25.1	0.12
10	1.5	193.0	26.5	0.13
11	2.0	243.5	27.1	0.14
12	2.0	193.0	30.0	0.18

Examples to obtain C/N ratios of 20 and 30 using Equation (1) stated in the methodology.

$$C/N = \frac{1.0 (34.74 * (100 - 0) + 294.0(4.31 * (100 - 94.20))}{1.0 (0.26 * (100 - 0) + 294.0(0.30 * (100 - 94.20))} = 20.0 \quad (2)$$

$$C/N = \frac{2.0 (34.74 * (100 - 0) + 193.0(4.31 * (100 - 94.20))}{2.0 (0.26 * (100 - 0) + 193.0(0.30 * (100 - 94.20))} = 30.0 \quad (3)$$

3.2. Kinetic of Biogas and Methane Yield

3.2.1. Effect of Carbon/Nitrogen (C/N) and Co-Substrate VS/Inoculum VS Ratios on Biogas Yield

Figure 6a presents the results of daily biogas yield for four reactors with low C/N ratios. The C/N ratios were 20.0, 21.1, 22.6, and 22.8, respectively. Each reactor contains 2 L of treated oily-biological sludge and 6.8, 8.21, 10.20, and 10.36 g of treated sugarcane bagasse, respectively. The biogas yield started from day 1 and increased gradually until day 12. Then it remains almost constant, with some fluctuations, until day 25. The biogas then started to slightly decrease from day 26 to the end of the reaction period, day 32. The rapid increase initially until day 12 may be due to the high availability of accessible organic matter from TOBS and TSCB as well. From day 13 to day 25, the biogas yields were almost

constant; this may due to the acclimatization of microorganisms with the substrates and operation environment. Meanwhile, there is still a high amount of organic matter to be consumed by the bacteria. However, the biogas yield slightly decreased from day 26 to the end of the reaction period; this indicated that most of the organic matter that can be consumed by the microorganisms under the current operation conditions was already consumed and the biogas yield started to decrease to the end of the reaction period.

Figure 6. (a,c,e): daily biogas yield, (b,d,f): cumulative biogas yield. C and d are the replicates of medium C/N ratio 24.2.

Figure 6b presents the cumulative biogas yield from the four reactors. The cumulative biogas yield was 2777, 3029, 2688, and 2822 mL, concurrent with 20, 21.1, 22.6, and 22.8 C/N ratios, respectively. The results indicate that the cumulative biogas yield from the reactor with 21.1 C/N is higher than cumulative biogas yield from the reactor with 20 C/N by 8.32%. In addition, the results indicate that the cumulative biogas yield from the reactor

with 22.8 C/N is higher than cumulative biogas yield from the reactor with 20 C/N by 1.59%. The increasing biogas yield may be due to volatile solid increment together with the C/N ratio. Overall, there is increasing of biogas yield together with C/N ratio increment. However, the behavior of biogas yield is still not clear and needs more investigation in terms of methane yield and increasing of C/N ratio.

Figure 6c shows the daily biogas yields from the central point 24.2 C/N ratio with four replicants. The behavior of daily biogas yield from the four reactors was quite similar. The biogas yield started from day 1 and increased gradually until day 14—this may be due to the high availability of degradable organic matter consumed by the microorganisms. After that, the biogas yield started to decrease from day 15 to day 18 and then remained constant with some fluctuations until day 29; this may due to a decrease in the organic matter content and the microorganisms acclimatized to the reaction environment. Then, a sharp decrease started from day 30 to day 32; this may be due to a sharp decrease in the available organic matter to be consumed by the microorganisms under the current operation conditions. When this C/N ratio is compared to previous C/N ratios, it is obvious that the duration of biogas' sharp increase as well as the constant period of biogas yield also increased. This may be due to the increasing of the C/N ratio by increasing the dosage of TSCB, as therefore the content of organic matter will increase, and the microorganisms will work more effectively.

Figure 6d shows the cumulative biogas yield from four reactors with the same C/N ratio, which is the central point 24.2. The cumulative biogas yields were 3638, 3459, 3863, and 3700 mL respectively. The average biogas yield is 3665 mL; this is higher than cumulative biogas yield from reactors with 20.0 and 22.8 C/N ratios by 24.23% and 23.0%, respectively. It is clear that the increasing biogas yield in this C/N ratio was enhanced greatly compared to the enhancement among the previous ratios. This indicates that the behavior of biogas is much clearer when increasing the C/N ratio by increasing the dosage of TSCB.

Figure 6e presents the daily biogas yield from the 25.1, 26.5, 27.1, and 30.0 C/N ratios. The behavior of biogas yield from the four reactors was quite similar. The biogas yield started increasing gradually from day 1 to day 7; this may be due to the high availability of degradable organic matter consumed by the microorganisms. After that, the 25.1 and 26.5 C/N ratio reactors almost remain constant, with a slight decrease and some fluctuations from day 8 to day 30, which may be due to a decrease in the organic matter content and the microorganisms acclimatized to the reaction environment. The 27.1 and 30.0 C/N ratio reactors remain constant, with a slight increase and some fluctuations from day 8 to day 30. This may be due to the acclimatization of microorganisms with the reaction environment and more availability of organic matter compared to 25.1 and 26.5 C/N reactors due to the increasing TSCB dosage. Then, a sharp decrease started from day 30 to day 32; this may due to the sharp decrease in the available organic matter to be consumed by the microorganisms under the current operation conditions. When these C/N ratios are compared to previous C/N ratios, it is obvious that the constant period of biogas yield increased. This may be due to increasing the C/N ratios to high levels by increasing the dosage of TSCB, as therefore the content of organic matter will increase and the microorganisms will work more effectively compared to lower volatile solids reactors.

Figure 6f shows the cumulative biogas yield from 25.1, 26.5, 27.1 and 30.0 C/N ratios. The cumulative biogas yields were 4867, 3683, 6808, and 9268 mL, respectively. The results indicate that the cumulative biogas yields from reactor with 30.0 C/N is higher than the cumulative biogas yields from reactors with 25.1, 24.2 and 20 C/N by 47.39%, 60.46%, and 70.04%, respectively. It is clear that increasing the biogas yield by gradually increasing the C/N ratio to higher levels is highly enhanced compared to the biogas yield from the lower C/N ratios. Overall, the yield of biogas increased gradually while increasing the C/N ratio by increasing the dosage of TSCB. This may be due to the fact that increasing the dosage of TSCB will result in increasing the volatile solids content. Therefore, the microorganisms' activity is enhanced and they are better acclimatized to the reaction environment, and the

biogas yield will enhance as well. In addition, at high C/N ratios between 25.1 and 30.0, the pH after 5 days of the reaction was stable between 6.9 and 7.3, which is within the ideal range of pH for better biogas production during anaerobic digestion, namely between 6.8–7.4, which provides a more healthy environment for bacteria [50,51]. Meanwhile, the lower C/N ratios cause ammonia inhabitation and the pH values increased and become toxic and inhibit the growth of methanogenic bacteria [52–55]. C/N ratios between 25 and 30 were also commonly found during previous studies to be better ratios during the anaerobic digestion process, with the C/N 30 ratio being the most common ratio [53,56–59], and this is consistent with the results of this study. Therefore, C/N 30 was selected from this study for the maximum biogas and methane yield and was validated using three replicates. The average after three-replicate validation of the maximum biogas yield from C/N 30 was found to be 9035.0 mL (195.6 mL /g VS_{removed}), with the significance level being more than 95%.

ANOVA Analysis for Biogas Yield from C/N 20–30

Appendix A, Table A1 summarizes the ANOVA analysis for 20.0–22.8 C/N ratios; it shows that there is no significant difference in the daily biogas yield, with a p value > 0.05 . This indicates that the dosage of TSCB added is still not enough to achieve a significant difference in the biogas yield from reactors with a low C/N ratio. Appendix A, Table A2 summarizes the ANOVA analysis for 20.0–24.2 C/N ratios; it shows that there is a significant difference in daily biogas yield, with a p value < 0.05 . This indicates that the dosage of TSCB added can cause a significant difference in the biogas yield from reactors when the C/N ratio increases to 24.2. Appendix A, Table A3 summarizes the ANOVA analysis for 25.1–30.0 C/N ratios; it shows that there is a significant difference in daily biogas yield, with a p value < 0.05 . This indicates that a larger dosage of TSCB added can achieve a highly significant difference in the biogas yield from reactors when the C/N ratio increases to 30.0. Appendix A, Table A4 summarizes the ANOVA analysis for 20.0–30.0 C/N ratios, and it shows that there is a significant difference in daily biogas yield, with a p value < 0.05 . This indicates that the overall increment for the dosage of TSCB added can achieve a significant difference in the biogas yield from reactors. Therefore, the C/N ratio is a key factor affecting the production of biogas; increasing the C/N ratio by adding more TSCB results in an increase in the biogas yield, and vice versa.

3.2.2. Effect of Carbon/Nitrogen (C/N) and Co-Substrate VS/Inoculum VS Ratios on Methane Yield

Figure 7a presents the results of the 3-day interval methane yield for four reactors with a low C/N ratio. The C/N ratios were 20.0, 21.1, 22.6, and 22.8, respectively. The measurement of the methane yield started from day 3 and the methane yield increased gradually until day 24, with some fluctuations. The methane content mainly related to the biogas yield quantity, as well the duration of reaction. When there was an increase in biogas yield, the methane content also increased and continued to gradually increase until day 24. Then, from day 25 to the end of the reaction, the methane yield sharply decreased; this may due to the fact that the biogas yield also sharply decreased as a result of the fact that most of the organic matter that can be consumed by the microorganisms under the current operation conditions was already consumed and the biogas yield started to decrease to the end of the reaction period.

Figure 7b presents the cumulative methane yield from the four reactors. The cumulative methane yield was 980.0, 1023.80, 1051.28, and 1067.28 mL, concurrent with 20, 21.1, 22.6, and 22.8 C/N ratios, respectively. The results indicate that the cumulative methane yield from the reactor with 21.1 C/N is higher than cumulative biogas yield from the reactor with 20 C/N by 6.78%. In addition, the results indicate that the cumulative biogas yield from reactor with 22.8 C/N is higher than cumulative biogas yield from reactor with 20 C/N by 8.18%. The increase in the methane yield is not high. However, the overall view of methane yield from these reactors shows that there is gradual increase in the methane yield together with the C/N ratio increment. The stability of the increase in the methane

yield is greater compared to the biogas yield. This may be due to the fact that the C/N ratio increment highly affects the methane yield compared to the biogas yield.

Figure 7. (a,c,e) Methane content yield every three days; (b,d,f) cumulative methane yield. c and d are the replicates of medium C/N ratio 24.2.

Figure 7c presents the results of the 3-day interval methane yield for four replicates of reactors with a central C/N ratio of 24.2. The measurement of methane yield started from day 3 and the methane yield increased gradually until day 15 with some fluctuations.

The methane content is mainly related to the biogas yield quantity as well the duration of the reaction. The biogas yield from day 16 until day 30 experienced a constant production rate with some fluctuations. Therefore, the methane yield was constant as well during this period. Then, the methane content from day 30 to the end of the reaction shows a sharp decrease in methane content, and this is mainly related to the sharp decrease noted in the biogas yield during this time.

Figure 7d presents the cumulative methane yield from the four replicate reactors. The cumulative methane yield was 1257.29, 1317.53, 1393.38, and 1317.57 mL, concurrent with the same C/N ratio of 24.2. The average methane yield is 1321.42 mL. The results indicate that the average cumulative methane yield from the reactor with 24.2 C/N is higher than the cumulative biogas yield from the reactor with 20 C/N by 25.84%. In addition, the results indicate that the average cumulative biogas yield from reactors with 24.2 C/N is higher than cumulative biogas yield from the reactor with 22.8 C/N by 19.24%. The increase in the methane yield from this ratio is high and promising compared to previous ratios.

Figure 7e presents the results of a 3-day interval methane yield for four reactors with a low C/N ratio. The C/N ratios were 25.1, 26.5, 27.1, and 30.0, respectively. The measurement of the methane yield started from day 3 and the methane yield increased gradually until day 15 with some fluctuations. The methane content was mainly related to the biogas yield as well the duration of reaction. The methane yield then experienced stability with some fluctuations until day 30. This may be due to the constant production of biogas during this period. Then, from day 30 to the end of the reaction, the methane yield sharply decreased due to the sharp decrease in the biogas yield.

Figure 7f presents the cumulative methane yield from the four reactors. The cumulative methane yield was 1617.18, 1723.40, 2007.84, and 3009.46 mL concurrent with 25.1, 26.5, 27.1, and 30.0 C/N ratios, respectively. The results indicate that the cumulative methane yield from the reactor with 25.1 C/N is higher than cumulative biogas yield from the reactor with 24.2 C/N by 18.29%. In addition, the results indicate that the cumulative biogas yield from the reactor with 30.0 C/N is higher than cumulative biogas yield from the reactor with 25.1 C/N by 46.26%. The results also indicate that the cumulative biogas yield from the reactor with 30.0 C/N is higher than the cumulative biogas yield from the reactor with 20.0 C/N by 67.44%. The increase in the methane yield is very high compared to previous C/N ratios. Overall, the methane yield was more stable and gradually increased in accordance with the C/N ratio increment compared to the biogas yield. The average of three-replicates validation for the maximum methane yield form C/N 30 was found to be 2996.07 mL (64.85 mL CH₄/g VS_{removed}), with a significance level of more than 95%.

The thermo-chemical pre-treatment of the sludge also contributes to the increased convertibility of the organic matter to biogas, which is similar to the observation by David C [60] during his study on the effect of thermal pre-treatment on waste activated sludge. He found that the increment of methane content increased concurrently with a temperature increment up to a maximum of 175 °C. Apart from that, different studies showed that the thermal and thermo-chemical pre-treatment increased the digestibility of the sludge during the anaerobic digestion process [61,62]. The thermo-chemical pre-treatment for sugarcane bagasse had a significant effect on enhancing biodegradability rate, which resulted in higher biogas production as well as methane content. This was also noticed by Costa [63] during his study on the effect of thermo-chemical pre-treatment to improve the anaerobic digestion of sugarcane bagasse.

ANOVA Analysis for Methane Yield

Although ANOVA analysis showed a non-significant increase in the methane yield during the lower increment of C/N ratios, as shown in Appendix B, Table A5, Table A6, and Table A7, there was a significant difference in the methane yield during the high increment of C/N ratios, as shown in Table A8, with a *p*-value of less than 0.05. This indicates that the increment of C/N and co-substrate VS/inoculum VS ratios has a higher effect on biogas yield than methane content. However, the methane yield production is more stable and

gradually increased together with C/N ratios increment without fluctuations compared to the biogas yield.

3.2.3. Effect of Carbon/Nitrogen (C/N) and Co-Substrate VS/Inoculum VS Ratios on Volatile Solids Removed

The VS removed increased progressively with C/N and co-substrate VS/inoculum VS ratios. According to Table 6, the minimum VS removed was met with minimum C/N and co-substrate VS/inoculum VS ratios. In contrast, the maximum VS removed was met with maximum C/N and co-substrate VS/inoculum VS ratios (30 and 0.18). The removal of volatile solids between minimum and maximum biogas and methane yields increased by 30.09% at C/N 30. The stable range of pH for all reactors was found to be between 6.9 and 8.0, and this was required for a proper environment for better microorganism activities. Therefore, the stability of a pH of 6.9–7.3, which is healthier for microorganisms at C/N 25.1–30, contributed to the higher removal of volatile solids and resulted in higher biogas and methane production. Alagöz and Maragkaki [64,65] indicated that VS removal improved during the co-digestion process for sludge with agricultural wastes, while the production of methane yields improved.

Table 6. Biogas yield and methane content vs. carbon/nitrogen and co-substrate VS/inoculum VS ratios.

Run No.	C/N	Volatile Solids Removed (g)	Biogas Yield (mL)	Methane Yield (mL)	Biogas Yield/VS Removed mL/g.VS _{removed}
1	20.0	32.3	2777	980.0	86.0
2	21.1	32.5	3029	1023.8	93.2
3	22.6	33.2	2688	1051.3	81.0
4	22.8	33.4	2822	1067.3	84.5
5	24.2	34.8	3638	1257.3	104.5
6	24.2	35.1	3459	1317.5	98.5
7	24.2	36.1	3863	1393.4	107.0
8	24.2	35.6	3700	1317.6	103.9
9	25.1	39.2	4867	1617.3	124.2
10	26.5	39.8	3683	1723.3	92.5
11	27.1	42.2	6808	2007.9	161.3
12	30.0	46.2	9268	3009.3	200.6

Table 7 shows the comparison of the current study with different studies used anaerobic co-digestion technology. This study shows better results than other studies in terms of biogas yield. However, the methane yield was lower than other studies. This may be due to the differences in materials' characteristics and co-digestion operational conditions. The present study indicated that anaerobic co-digestion using a co-substrate with a high C/N ratio and VS content to balance the lower C/N ratio of the oily-biological sludge would enhance the digestibility, resulting in higher biogas and methane yields in case we used mono-digestion. However, to increase the methane yield from oily-biological sludge with sugarcane bagasse, the pre-treatment method or parameters for both materials can be changed and tested. For instance, the delignification process for sugarcane bagasse using thermo-chemical method can be optimized using the response surface methodology to minimize the lignin content. In addition, another pre-treatment method can be applied. In addition, for the oily-biological sludge, the pre-treatment parameters and method can also be changed. For example, we can increase the temperature and reduce the time when using thermo-chemical method, or use a different pre-treatment method such as ultrasonic pre-treatment. In addition, we can test the methane yield by changing the operational condition during the anaerobic co-digestion process or by changing the type of digestion

method. For instance, we can change the temperature from a mesophilic thermophilic condition or decrease the mixing speed during the co-digestion process. In addition, changing the type of digestion method could enhance the methane yield, such as using the two-stage anaerobic co-digestion method.

Table 7. Various substrates to enhance biogas yields during the anaerobic co-digestion process.

Substrate	Co-Substrate	Biogas and Methane Yield	Digestion Time (Batch)	Reference
Oil refinery wastewater	Sugarcane bagasse	154.72 mL/g VS, 97.13 mL of CH ₄ /g VS	34 days	[66]
Waste activated sludge	Grape pomace	180 mL/gVS _{removed} , 110 mL of CH ₄ /gVS _{removed}	30 days	[67]
Oil refinery biological sludge	Sugarcane bagasse	200.6 mL/g VS _{removed} , 63.52 mL of CH ₄ /gVS _{removed}	33 days	This study

4. Conclusions

In this study, sugarcane bagasse was used as a high C/N ratio co-substrate to treat the low C/N ratio oily-biological sludge through anaerobic co-digestion technology. Thermo-chemical pre-treatment using NaOH was carried out for sugarcane bagasse to reduce the lignin content as well as to increase the pH. The best ratio for the sugarcane delignification process as a part of pre-treatment was 1% (*w/v*) sodium hydroxide, 1:10 solid-liquid ratio, at 100 °C, and 150 rpm for 1 h. In addition, thermo-chemical pre-treatment were carried out for the oily-biological sludge using NaOH to increase the sludge digestibility and to increase the pH as well. The gradual increase in the C/N ratio resulted in a gradual rise in sugarcane bagasse content along with co-substrate volatile solids/inoculum volatile solids. Therefore, the biogas yield and methane content increased when higher C/N and co-substrate VS/inoculum VS were used. The biogas yield from C/N 30 was higher than the biogas yield from C/N 20 by 70.04%. Meanwhile, the methane yield from C/N 30 was higher than the methane yield from C/N 20 by 67.44%. The maximum biogas and methane yield coincided with the highest C/N and co-substrate VS/inoculum VS ratios (30.0 and 0.18) with 200.6 mL/g VS_{removed} and 65.1 mL CH₄/g VS_{removed}, respectively. This increase was attributed to the increment of volatile solids content while increasing the C/N ratios. In addition, it was due to the stability of pH for high C/N ratios reactors between 6.9 and 7.3, which is more healthy for microorganisms activities. The lower C/N ratios cause an increased ammonia concentration, which is toxic to the bacteria, resulting in a lower biogas and methane yield.

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Data Availability Statement: This project still on going and the data cannot be published at the current stage because there might be more analysis and comparison of the data after anaerobic co-digestion process under thermophilic condition. However, in case any researcher needs the data can either contact us via email or use Digitizer Plot for the graphs to extract the data.

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Novelty Statement: To the authors' best knowledge, there is no published analysis of the anaerobic co-digestion of oily-biological sludge generated from petroleum refineries' wastewater treatment system with sugarcane bagasse under mesophilic or thermophilic conditions. This study used mesophilic anaerobic co-digestion technology. Both mechanical and thermo-chemical pre-treatment methods were applied to the raw materials prior to the digestion process to enhance the digestibility and improve microorganism activities by reducing the lignin content of sugarcane bagasse and increasing the sludge solubilization and stabilization. Due to the acidic nature of the sludge and sugarcane bagasse, NaOH was used during the pre-treatment process to adjust the pH during the anaerobic co-digestion process within 6–8. The design for the batch mixtures was determined based on C/N ratios and co-substrate VS/inoculum VS ratios to analyse their effects on biogas yield, methane content, and VS removed. A part of this project was recently published in the *Sustainability* journal, which contains the prediction of methane yield from this design [32]. However, this study focused and extended on the analysis, the characterization of thermo-chemically pre-treated materials, as well as the kinetics of daily biogas yields, along with interval methane yields using one-way ANOVA analysis, and the effects of C/N ratios with co-substrate VS/inoculum VS ratios on total biogas and methane yield.

Appendix A

Table A1. One-way (ANOVA) test for daily biogas yield from the first four reactors.

Anova: Single Factor SUMMARY						
Groups	Count	Sum	Average	Variance		
C/N = 20.0	32	2777	86.78125	923.1442		
C/N = 21.1	32	3029	94.65625	2288.426		
C/N = 22.6	32	2688	84	1946.71		
C/N = 22.8	32	2822	88.1875	428.3508		
ANOVA						
Source of Variation	SS	df	MS	F	p-value	F crit
Between Groups	1957.313	3	652.4375	0.467142	0.705731	2.677699
Within Groups	173,185.6	124	1396.658			
Total	175,142.9	127				

Table A2. One-way (ANOVA) test for daily biogas yield from the first five reactors.

Anova: Single Factor SUMMARY						
Groups	Count	Sum	Average	Variance		
C/N = 20.0	32	2777	86.78125	923.1442		
C/N = 21.1	32	3029	94.65625	2288.426		
C/N = 22.6	32	2688	84	1946.71		
C/N = 22.8	32	2822	88.1875	428.3508		
Ave C/N = 24.2	32	3665	114.5313	1083.051		
ANOVA						
Source of Variation	SS	df	MS	F	p-value	F crit
Between Groups	19,429.71	4	4857.428	3.641424	0.007262	2.430002
Within Groups	206,760.2	155	1333.936			
Total	226,189.9	159				

Table A3. One-way (ANOVA) test for daily biogas yield from the last four reactors.

Anova: Single Factor SUMMARY						
Groups	Count	Sum	Average	Variance		
C/N = 25.1	32	4867	152.0938	3291.184		
C/N = 26.5	32	3683	115.0938	2190.152		
C/N = 27.1	32	6808	212.75	7365.226		
C/N = 30.0	32	9268	289.625	30,224.18		
ANOVA						
Source of Variation	SS	df	MS	F	p-value	F crit
Between Groups	558,965.5	3	186,321.8	17.3038	1.9×10^{-9}	2.677699
Within Groups	1,335,193	124	10,767.68			
Total	1,894,158	127				

Table A4. One-way (ANOVA) test for daily biogas yield from all reactors.

Anova: Single Factor SUMMARY						
Groups	Count	Sum	Average	Variance		
C/N = 20.0	32	2777	86.78125	923.1442		
C/N = 21.1	32	3029	94.65625	2288.426		
C/N = 22.6	32	2688	84	1946.71		
C/N = 22.8	32	2822	88.1875	428.3508		
Ave C/N = 24.2	32	3665	114.5313	1083.051		
C/N = 25.1	32	4867	152.0938	3291.184		
C/N = 26.5	32	3683	115.0938	2190.152		
C/N = 27.1	32	6808	212.75	7365.226		
C/N = 30.0	32	9268	289.625	30,224.18		
ANOVA						
Source of Variation	SS	df	MS	F	p-value	F crit
Between Groups	1,271,971	8	158,996.4	28.76871	1.61×10^{-32}	1.971665
Within Groups	1,541,953	279	5526.714			
Total	2,813,924	287				

Appendix B

Table A5. One-way (ANOVA) test for methane yield every three days from the first four reactors.

Anova: Single Factor SUMMARY						
Groups	Count	Sum	Average	Variance		
C/N = 20.0	11	979.9306	89.0846	3286.609		
C/N = 21.1	11	1023.803	93.07296	2818.605		
C/N = 22.6	11	1051.351	95.57733	7029.023		
C/N = 22.8	11	1067.176	97.01602	1813.93		
ANOVA						
Source of Variation	SS	df	MS	F	p-value	F crit
Between Groups	398.3631	3	132.7877	0.035533	0.990889	2.838745
Within Groups	149,481.7	40	3737.041			
Total	149,880	43				

Table A6. One-way (ANOVA) test for methane yield every three days from the first five reactors.

Anova: Single Factor SUMMARY						
Groups	Count	Sum	Average	Variance		
C/N = 20.0	11	979.9306	89.0846	3286.609		
C/N = 21.1	11	1023.803	93.07296	2818.605		
C/N = 22.6	11	1051.351	95.57733	7029.023		
C/N = 22.8	11	1067.176	97.01602	1813.93		
Ave C/N = 24.2	11	1321.423	120.1293	3433.406		
ANOVA						
Source of Variation	SS	df	MS	F	p-value	F crit
Between Groups	6550.961	4	1637.74	0.445484	0.775136	2.557179
Within Groups	183,815.7	50	3676.314			
Total	190,366.7	54				

Table A7. One-way (ANOVA) test for methane yield every three days from the last four reactors.

Anova: Single Factor SUMMARY						
Groups	Count	Sum	Average	Variance		
C/N = 25.1	11	1617.179	147.0163	5369.843		
C/N = 26.5	11	1723.404	156.6731	12,032.22		
C/N = 27.1	11	2007.842	182.5311	13,639.7		
C/N = 30.0	11	3009.458	273.587	31,249.45		
ANOVA						
Source of Variation	SS	df	MS	F	p-value	F crit
Between Groups	110,009.3	3	36,669.78	2.354732	0.086381	2.838745
Within Groups	622,912.2	40	15,572.8			
Total	732,921.5	43				

Table A8. One-way (ANOVA) test for methane yield every three days from all reactors.

Anova: Single Factor SUMMARY						
Groups	Count	Sum	Average	Variance		
C/N = 20.0	11	979.9306	89.0846	3286.609		
C/N = 21.1	11	1023.803	93.07296	2818.605		
C/N = 22.6	11	1051.351	95.57733	7029.023		
C/N = 22.8	11	1067.176	97.01602	1813.93		
Ave C/N = 24.2	11	1321.423	120.1293	3433.406		
C/N = 25.1	11	1617.179	147.0163	5369.843		
C/N = 26.5	11	1723.404	156.6731	12,032.22		
C/N = 27.1	11	2007.842	182.5311	13,639.7		
C/N = 30.0	11	3009.458	273.587	31,249.45		
ANOVA						
Source of Variation	SS	df	MS	F	p-value	F crit
Between Groups	318,877.3	8	39,859.66	4.446814	0.000144	2.042986
Within Groups	806,727.9	90	8963.643			
Total	1,125,605	98				

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