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Exploration of One-Factor Rsm to Optimize the Concentration of Organic Fraction of Municipal Solid Waste (OFMSW) for Biogas Production

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Abstract

The problem of pollution resulting improper management of municipal solid waste (MSW) in Nigeria needs to be eliminated by converting MSW to useful resources. In this study, we conducted lab-scale anaerobic digestion of OFMSW to optimize substrate concentration required to maximize biogas yield under wet ambient condition. After characterization, various concentrations of the substrate (OFMSW) ranging from 0% (wet process) to 45% (dry process) was subjected to One-Factor response design (using Design Expert version 9.0) as well as anaerobic digestion (using rumen juice as the source of microbial inoculum) inside one-stage 500 ml-capacity batch-type anaerobic digesters with useful volumes of 350 ml. Result showed that the highest and lowest volume of cumulative biogas production (596.4 ml and 107.6 ml) was recorded in the experimental set-up with 30% and 5% substrate respectively after 42days. However, the highest biogas yield (8.51 ml/gr. VS) was recorded in the experimental set- up with 5% substrate followed by the experimental set-up with 30% substrate (7.86 ml/gr. VS), while the lowest biogas yield (0.96 ml/gr. VS) was recorded in the experimental set-up with 45% substrate. Analysis of the response surface design showed that the optimum substrate concentration required to maximize biogas yield (~ 8.66 ml/gr. VS) in the wet process under ambient (lab) condition was approximately 5.52%. Confirmatory test for anaerobic digestion of the predicted optimum substrate concentration (5.52%) produced an average biogas yield of 7.03+1.453 ml/gr. VS. This result suggests that the true biogas yield under this wet process may lie between 5.58 ml/gr. VS and 8.48 ml/gr. VS. Finally, we isolated and identified bacteria species belonging to genera such as *Bacillus, Bacteroides, Clostridium, Enterobacter, Escherichia, Lactobacillus, Micrococcus, Morganella, Propionibacterium, Pseudomonas, Providencia, Ruminococcus, Staphylococcus and Streptococcus* inside the rumen juice, substrate and composite sample of the digestate respectively.

Keywords: Optimization; Substrate concentration; Anaerobic digestion; Biogas yield

Introduction

Waste management has become an issue of growing global concern as urban populations continue to increase and consumption patterns change. The health and environmental implications associated with garbage disposal are mounting in urgency, particularly in developing countries like Nigeria. Anaerobic digestion of organic fraction of municipal solid waste (OFMSW) is of great importance in the management of solid waste and by application, it will considerably decrease the volume of waste that is being generated. On the other hand, as one of the driving forces of economic and social development, anaerobic digestion of OFMSW for the production of sustainable biofuel (such as biogas) as well as biofertilizer has become a growing world interest [1-13]. The initial concentration or solid content of the substrate in a bioreactor can significantly affect performance of the anaerobic digestion process [14-19]. In order to increase the efficiency of anaerobic digestion, it is necessary to understand the role of total solids content (substrate concentration) on the behaviour of the microbial communities involved in anaerobic digestion of organic matter in wet and dry technology [18]. Low solid anaerobic digestion systems contain less than 10% total solid, medium solid anaerobic digestion systems contain around 15% to 20% total solid and high solid anaerobic digestion systems contain around 22% to 40% total solid of the substrate [10]. Ordinarily solid concentrations between 6% and 10% are said to be best suited for biogas production under wet anaerobic digestion condition [20-22]. As part of a pilot scale study, we conducted a laboratory-scaled anaerobic digestion of organic fraction of municipal solid waste (OFMSW) to determine the optimum substrate concentration required to maximize biogas yield under wet ambient condition.

Materials and Methods

Laboratory scale anaerobic digester set-up

For each experimental set-up, one-stage 500ml-capacity anaerobic digestion (AD) system was configured for batch-type mesophilic process with useful volume of around 350 ml (Figure 1). The first (500 ml capacity) plastic bottle served as the anaerobic digester where biogas was generated. The second (250 ml capacity) conical flask connected to a 1 m long glass tube contained water that had been saturated with salt (NaCl) to prevent the incoming biogas (generated inside the plastic bottle) from dissolving inside the water. The small balloon (which was connected to the conical flask through a mini rubber hosepipe) served as the collection chamber for the biogas produced. When biogas enters the conical flask, it displaces an equal volume of water which rises through the glass tube. To estimate the volume of biogas produced, the height of water displaced by the biogas is measured using a meter rule and applied to the formula shown in equation one to calculate the volume of water displaced. In other words, the volume of water displaced was taken to be equivalent to the volume of biogas produced [18] (Equation 1).

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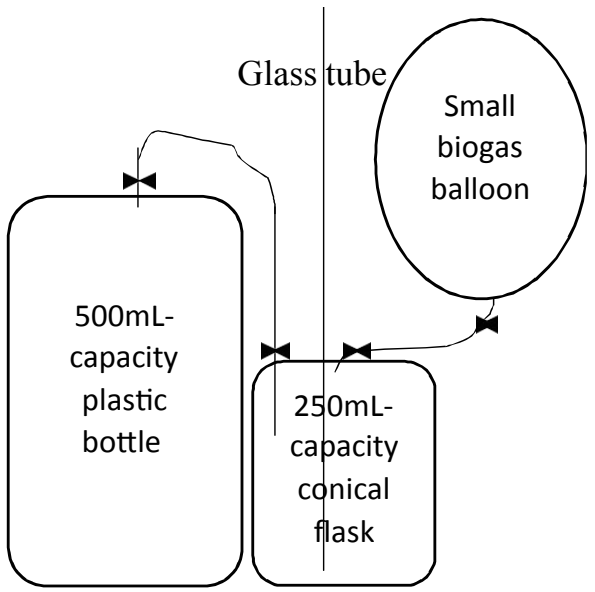

Figure 1: Set-up for laboratory – scale anaerobic digestion of municipal solid waste.

$$V = \pi r2h \quad \text{(Eq. 1)}$$

Where, V=Volume, r=radius of the glass tube, h=height of water displaced and π=3.142857.

Laboratory scale experimental design

To determine the optimum substrate concentration (%) that will maximize the rate of biogas production under wet ambient condition, we subjected various concentrations of the substrate (OFMSW) ranging from 0% (wet process) to 45% (dry process) to One-Factor response design (using Design Expert version 9.0), which produced a total of ten runs as shown in Table 1. The independent variable selected for this study was substrate concentration (%) while the dependent variable (i.e., the response) selected was biogas yield (ml/gr. VS).

Preparation of rumen juice (rj)

Cow's rumen juice (as the source of microbial inoculum) was obtained and prepared as described by Ogbonna et al. [12]. The filtered rumen juice was transferred into a 2 L-capacity gallon and supplemented with 20 g of glucose. This was done in order that the microbes trapped inside the juice would generate more energy from utilizing glucose as substrate to breakdown any complex organic polymer (such as cellulose) which may have been retained in the rumen juice after filtration. Following this, the rumen juice was injected with 1.8 ml of $Na_2S.9H_2O$ (2% w/v) using a long needle attached to a 10 ml syringe and the gallon was screw capped with a specially designed cap which allowed us to evacuate biogas from the 2 L-capacity gallon with time (Figure 1). Addition of hydrated sodium sulphide was done to reduce the rumen juice in order to promote the growth of strict anaerobic bacteria trapped inside the juice. Following this, the populations of aerobic and anaerobic bacteria were determined by cultural enumeration before and after subjecting the rumen juice to anaerobic digestion in the dark under ambient (laboratory) condition until biogas production was no longer observed (two month later).

Collection and pre-treatment of municipal solid waste

Paper waste was collected at source in Oba market, Benin City (Nigeria). After collection, the paper waste was shredded using paper shredder. The shredded paper waste was transferred into a pressure pot containing water and boiled for three hours. After boiling, we allowed the paper-water mixture to stand for two weeks. Thereafter, we removed the excess water by filtration using a textile filter and sun-dried the heat-treated paper waste. After drying, the paper was milled into powdered form using a grinding machine and preserved in a nylon bag. These treatment procedures were applied in to increase the biodegradability of the paper waste. Due to the high biodegradability of some municipal solid waste such as food waste, fruit waste, vegetable waste, etc., we delayed their collection until we were ready to formulate the feed so as to prevent excessive loss of volatile solid if kept for long period. These fractions of municipal solid waste was collected at source from Oba market in Benin City Edo State (Nigeria) using waste collection bags. After collection, the wastes were pooled and milled together to produce a pasty homogeneous solid. Milling reduces particle size of the substrate, thus making it more bioavailable to the microbes [18].

Preparation and characterization of the substrate

The substrate was prepared by mixing the powdered paper waste with the pasty solid derived from the pre-treatment of other organic fraction of municipal solid waste to form the wet solid substrate. After this, samples of the substrate were collected to determine some of its physical properties such as dry (or total) solid (TS), water content (WC), volatile solid (VS) and ash content (AC) using the method of USEPA [23]. From the samples collected, the populations of aerobic and anaerobic bacteria were determined via cultural enumeration.

Preparation and anaerobic digestion of the feed

The feed in each anaerobic digestion set-up was formulated to arrive at the desired substrate concentration (%) shown in Table 1 using the formula in Equation two (2). Anaerobic digestion of the feed was carried out inside the 500 ml-capacity plastic bottle shown in Figure 2 under ambient (laboratory) condition with a retention time of 42 days. During the process, biogas production in each set-up was measured volumetrically using the water displacement technique described above (Figure 2). Ambient temperature was also measured using digital thermometer (SCT-lilliput, Scichem Tech) with time.

$$\text{Solid content}(\%) = \frac{\text{Mass of dry OFMSW} \times 100}{\text{Mass of dry OFMSW + volume of rumen juice + Volume of } H_2O} \quad (2)$$

After modelling the rate of biogas production (i.e., biogas yield) with respect to substrate concentration (%), confirmatory experiment was conducted (in triplicates) in order to compare the observed biogas yield and the predicted solution for biogas yield, which was generated by the One-Factor RSM.

Enumeration and isolation of aerobic and anaerobic bacteria

Bacteria populations were determined based on oxygen requirement inside the rumen juice, pre-treated substrate (OFMSW) and composite digestate (a pool of the digestates from all ten set-ups) to confirm their presence. Aerobic bacteria were enumerated and isolated as described by Abdulkadir and Waliyu [24]. Strict anaerobic bacteria were enumerated and isolated using the agar roll-tube technique described by Holdeman and Moore [25] and Wolfe [26] respectively.

Identification of aerobic and anaerobic bacterial isolate

Bacterial isolates were identified according to Bergey's Manual of Determinative Bacteriology [27] and Bergey's Manual of Systemics of archaea and Bacteria [28] using morphological and metabolic/biochemical tests. These bacteriological characterization tests included

Std	Run	Substrate (%)	DS(g)	VS(g)	WC(g)	*WS (g)	*RJ (ml)	*WA (ml)	Total (ml)
4	1	**15**	52.5	37.9	9.5	62.0	26.3	261.7	350
6	2	**35**	122.5	88.50	20.4	142.9	61.3	145.9	350
3	3	**5**	17.5	12.64	3.2	20.7	8.8	320.5	350
5	4	**30**	105.0	75.90	19.0	124.0	52.5	173.5	350
10	5	**20**	70.0	50.60	12.6	82.6	35.0	232.4	350
1	6	**0**	0.0	0.0	0.0	0.0	0.0	350.0	350
8	7	**45**	157.5	113.80	28.4	185.9	78.8	85.3	350
2	8	**0**	0.0	0.0	0.0	0.0	0.0	350.0	350
7	9	**45**	157.5	113.80	28.4	185.9	78.8	85.3	350
9	10	**20**	70.0	50.60	12.6	82.6	35.0	232.4	350

Table 1: Actual design by one-factor RSM for analysis of biogas production rate.

Gram staining, acid fast staining, motility test, oxygen requirement test, oxidase test, catalase test, coagulase test, citrate test, indole test, urease test, hydrogen sulphide production, nitrate reduction, Methyl red test, Voges Proskauer test, ornithine decarboxylase test, glucose fermentation, mannitol fermentation, sucrose fermentation, lactose fermentation, maltose fermentation, xylose fermentation, arabinose fermentation, salicin fermentation, cellobiose fermentation, mannose fermentation, melezitose fermentation, raffinose fermentation, sorbitol fermentation, trehalose fermentation, glycerol fermentation, cellulose hydrolysis, starch hydrolysis, gelatin hydrolysis and esculin hydrolysis respectively.

Statistical analysis

Using Design Expert (DX version 9.0) software, we subjected the data of one-factor response designs in Table 1 to regression analysis in other to obtain the parameters required for modelling the rate of biogas production with respect to substrate concentration. With MS Excel (2013), 2-way ANOVA was employed to determine if there was a significant difference in cumulative biogas production with respect to substrate concentration and time.

Results and Discussion

Physical properties of the substrate (pre-treated MSW)

Water content, dry (or total) solid content, ash content and volatile solid content of the substrate was 15.29%, 84.71%, 27.76 and 72.24% respectively. The volatile solid content of the substrate is a measure of the substrate biodegradability [18]. Generally, substrate degradability between the range of 0% to 20% may be bio-recalcitrant, substrate degradability between 20% – 70% may either be very slowly biodegradable or moderately biodegradable while substrate degradability greater than 70% is said to be readily (or easily) biodegradable. Our substrate can be said to be easily biodegradable because it had a biodegradability of approximately 72.24%. The volatile solid of 72.24% meant that about 72.24% of the substrate was biodegradable at the time. Therefore, approximately 72.24% of the dry substrate should be converted to biogas when subjected to anaerobic digestion. Average ambient temperature (°C) during the laboratory-scale anaerobic digestion of OFMSW ranged from 25°C to 32°C with time (Figure 2). This temperature range actually lie within the operational mesophilic temperature requirement (i.e., 20°C to 45°C) for biogas production with the optimum said to be between 35°C and 37°C [18,29].

Cumulative biogas production

In the control set-up (with 0% substrate concentration), there was no biogas production (Figure 3). In the experimental set-ups with 5%, 15%, 20% and 20*% substrate concentrations, cumulative biogas production increased to 107.6 ml, 167.4 ml, 232.8 ml and 212.5 ml respectively with time (Figures 4-7). In the experimental set-ups with 30%, 35%, 45% and 45% substrate concentration, cumulative biogas production increased to 596.4 ml, 384.1 ml, 131.8 ml and 109.2 ml respectively with time (Figures 8-11). Figures 4-11 show that cumulative biogas production tend to resemble the sigmoid function (S curve) as generally observed in microbial batch growth [30]. This was expected because biogas production rate under batch condition directly corresponds to bacterial population growth dynamics (especially the methanogens) during anaerobic digestion of organic matter [12,17,30,31]. The highest volume of cumulative biogas production (596.4 ml) was recorded in the experimental set-up with 30% substrate concentration (Figure 12). However, the highest rate of biogas production or biogas yield (8.51 ml/gr. VS) was recorded in the experimental set-up with 5% substrate concentration followed by the experimental set-up with 30% substrate concentration with a biogas yield of 7.86 ml/gr. VS (Figure 13). This result suggested that the optimum substrate concentration (%) required to maximize biogas yield in wet and dry processes may lie around 5% and 30% respectively (Figure 13). The 2-Way ANOVA in Table 2 shows that there was a significant difference in cumulative biogas production (ml) with respect to the variation in substrate concentration (%) with time.

Analysis of the response surface design

Fit-summary of the design (Table 3), sequential sum of squares (Table 4), lack of fit tests (Table 5) and model summary statistics (Table 6) suggested that the response surface fifth (5^{th}) and response surface sixth (6^{th}) models would be adequate in describing biogas production rate (ml/gr. VS) with respect to substrate concentration (%) under the condition of operation because the low p-values ($P<0.05$) of both models indicated a highly significant advantage over the other models. Both models had near perfect R^2 (0.9938 and 0.9861) and adjusted R^2 (0.9988 and 0.9964) respectively. The sixth model was selected because it had the highest R^2 (0.9988) and adjusted R^2 (0.9964) as well as the lowest standard deviation (0.18) which is a good measure of its relative precision for forecasting outcomes (Table 6). ANOVA for the Response Surface Sixth Model shown in Table 7 indicated that the regression (6^{th}) model fitted to the response (i.e., biogas yield) is statistically significant (at $p<0.05$). The adjusted R^2 (0.9964) showed that this response surface 6^{th} model could adequately explain about 99.64% of variation in the rate of biogas production with respect to substrate concentration (Table 7). The final equation generated by the model for predicting biogas yield (ml/gr VS) with respect to substrate concentration (%) is shown in equation three.

$$Y=3.86023E\text{-}013 + 3.38826X - 0.41100X^2 + 0.014594X^3 + 8.98704E\text{-}005X^4 - 1.25163E\text{-}005X^5 + 1.55605E\text{-}007X^6$$ (Equation 3). Where Y=Rate of biogas production (ml/gr. VS) and X=Substrate concentration (%)

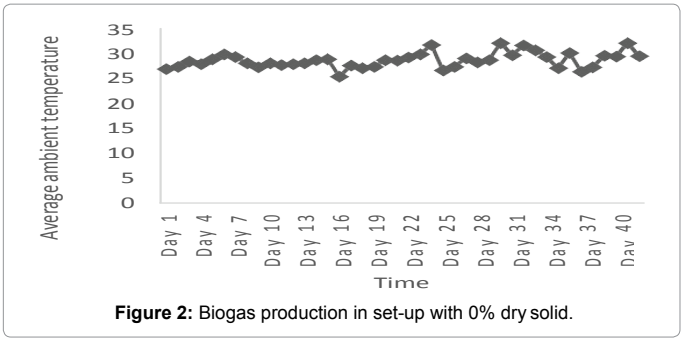

Figure 2: Biogas production in set-up with 0% dry solid.

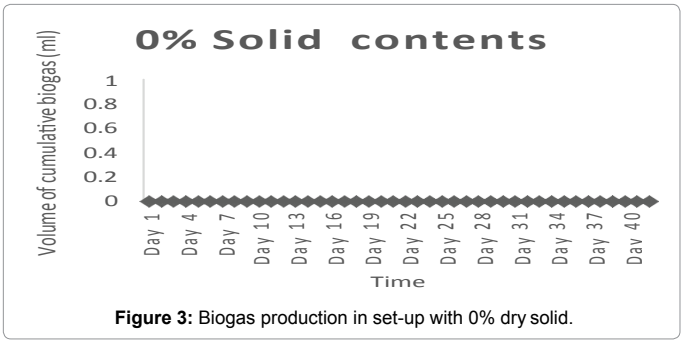

Figure 3: Biogas production in set-up with 0% dry solid.

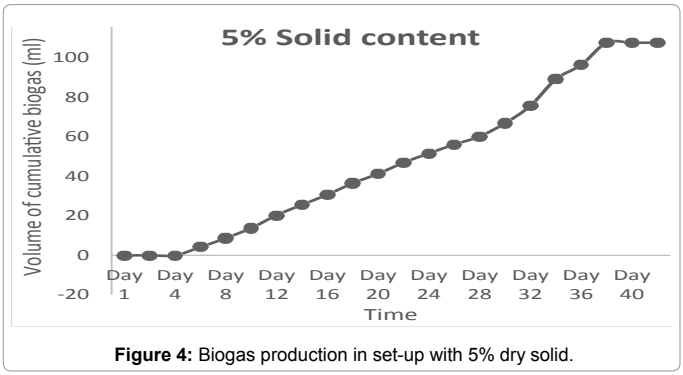

Figure 4: Biogas production in set-up with 5% dry solid.

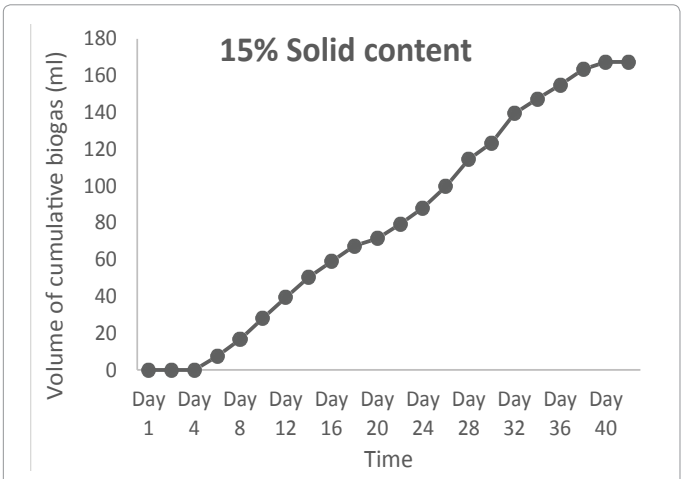

Figure 5: Biogas production in set-up with 15% dry solid.

Effect of plot and response optimization

The response surface plot presented in Figure 14 shows how biogas yield (ml/gr. VS) varied as a function of substrate concentration (%). The

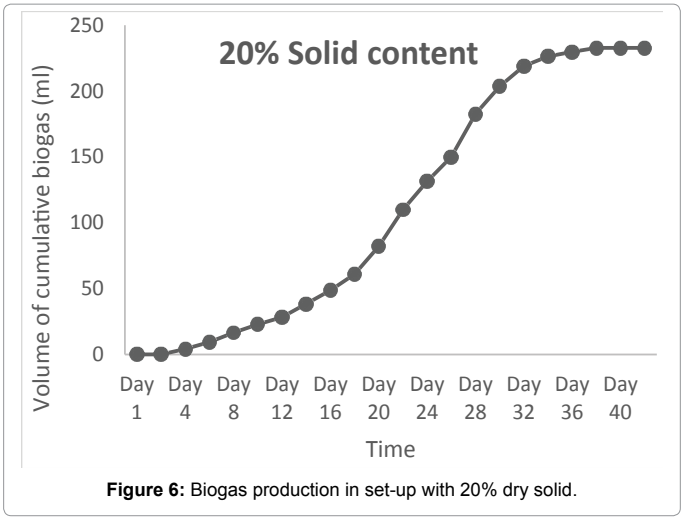

Figure 6: Biogas production in set-up with 20% dry solid.

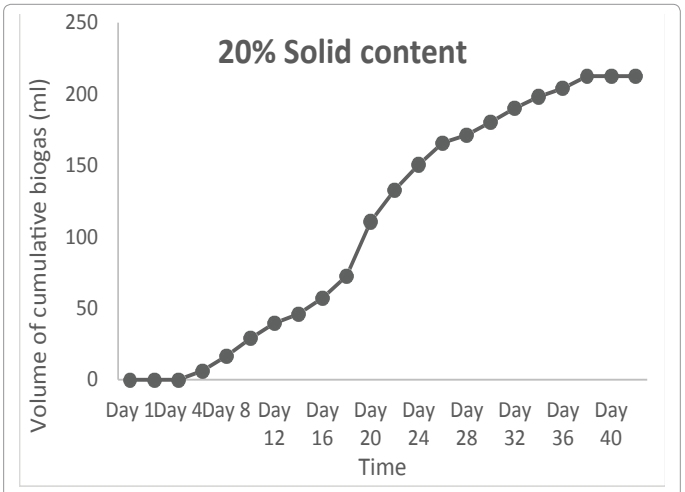

Figure 7: Biogas production in set-up with 20% dry solid

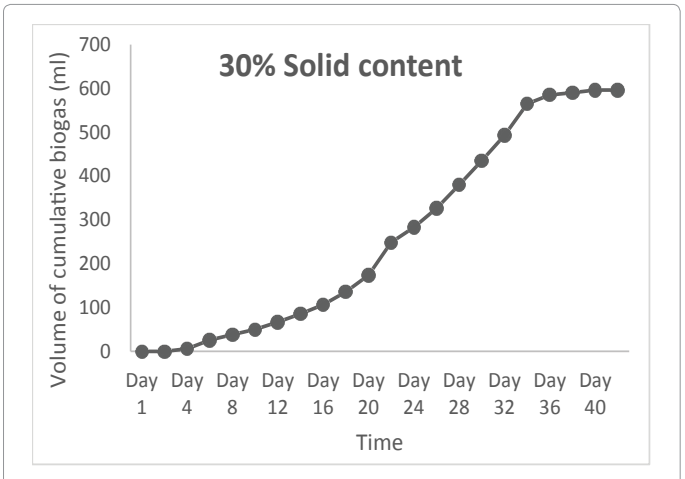

Figure 8: Biogas production in set-up with 30% dry solid.

dotted lines represent the 95% confidence band on the mean prediction at any given substrate concentration (%). The solid line represents the mean prediction (according to the model shown in equation 3) for the rate of biogas production with respect to substrate concentrate (%). The points on the response surface plot represent the actual response

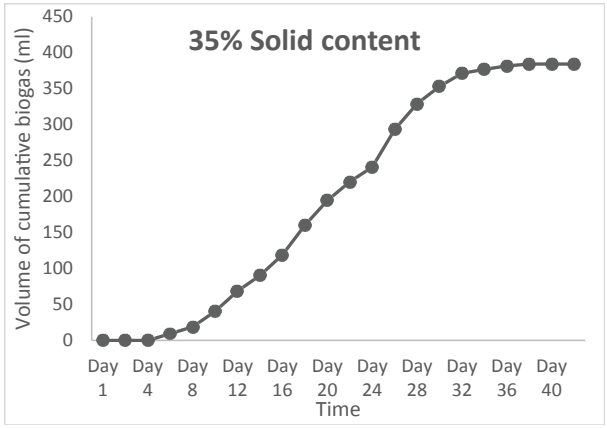

Figure 9: Biogas production in set-up with 30% dry solid.

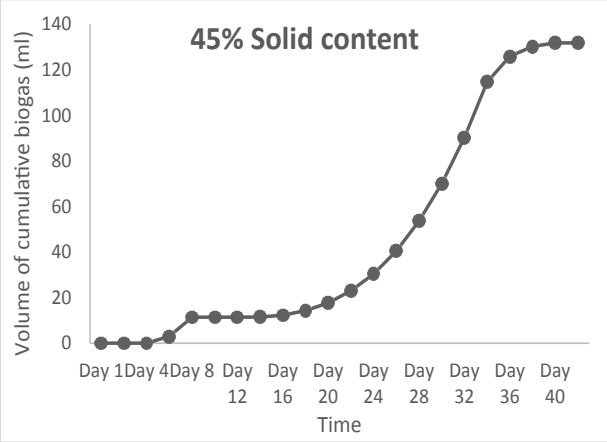

Figure 10: Biogas production in set-up with 30% dry solid.

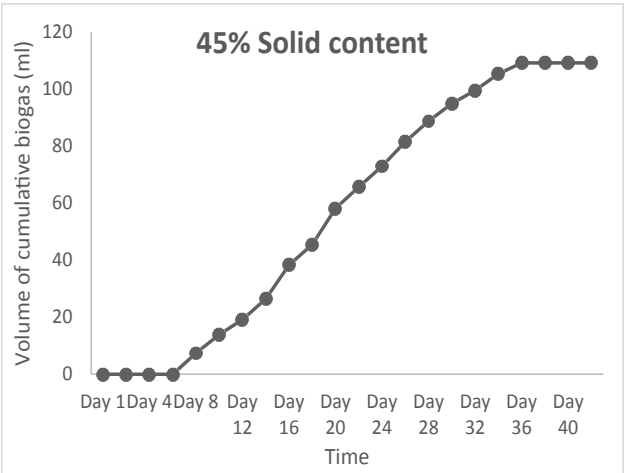

Figure 11: Biogas production in set-up with 35% dry solid.

which has already been presented in Figure 13. In the actual response, the highest biogas yield (8.51 ml/gr. VS) was observed at 5% substrate concentration (Figure 13) and the standard error was relatively low (~ 0.18). However, the optimum response generated showed that the substrate concentration required to maximize biogas yield (~ 8.66 ml/gr. VS) under the wet condition of operation was approximately 5.52%

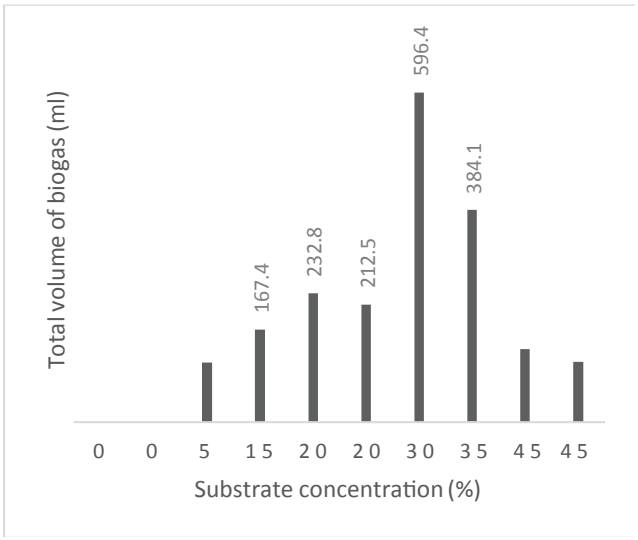

Figure 12: Volume of cumulative biogas (ml) production.

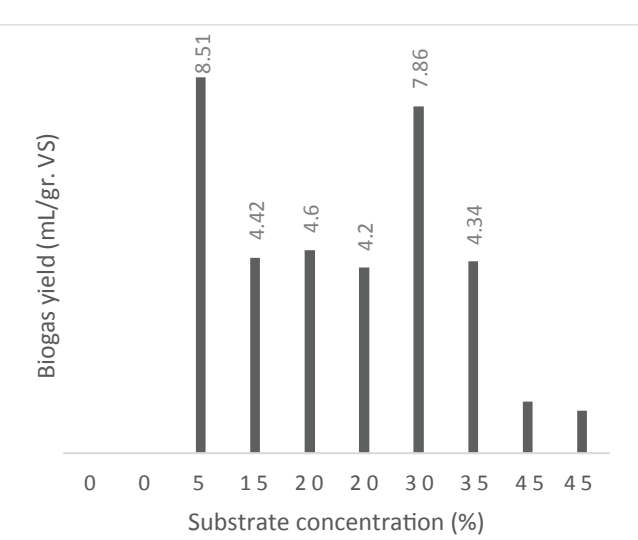

Figure 13: Rate of biogas production or biogas yield (ml/gr. VS).

with a standard error of approximately 0.17 and a desirability of 0.375 respectively (Figure 15). The confirmatory test for anaerobic digestion of the predicted optimum substrate concentration (5.52%) which was performed in triplicates under ambient (lab) condition gave an average biogas yield of 7.03 ± 1.453 ml/gr. VS at $p<0.05$. This result suggests that the true biogas yield under the condition which the wet process occurred may lie between 5.58 ml/gr. VS and 8.48 ml/gr. VS. This is not far from the initial actual response and the predicted optimum presentedabove. Several research reports have shown that the optimum substrate concentration (%) required for maximizing the rate of biogas production in wet anaerobic digestion of various forms of organic matter (including municipal solid waste) may lie between 4% and 10% depending on the condition of operation [15,18,19,21,32]. Our result of optimum substrate concentration (at 5.52%) for biogas production rate under ambient lab conditions lies within this range. This could be as a result of the fact that substrate concentration below 4% and above 10%

Source of Variation	SS	df	MS	F_{cal}	P-value	F crit
Substrate conc. (%)	1130889	8	141361.1	29.79282**	1.32E-28	1.993884
Time (days)	1414531	21	67358.61	14.19629**	2.69E-27	1.619182
Error	797127	168	4744.804			
Total	3342546	197				

Table 2: 2-way ANOVA of cumulative biogas (ml).

Source	Sequential p- value	Lack of Fit p- value	Adjusted R- Squared	Predicted R- Squared	
Linear	0.9666	0.0001	-0.1247	-0.6996	
Quadratic	0.0394	0.0003	0.3278	0.0947	
Cubic	0.8677	0.0002	0.2197	-0.2247	
Quartic	0.0437	0.0004	0.6161	-0.7483	
Fifth	0.0003	0.0383	0.9861	0.7412	Suggested
Sixth	0.0383		0.9964		Suggested

Table 3: Fit summary (detailed tables shown below).

Source	Sum of Squares	df	Mean Square	F-Value	p-value Prob > F	
Mean vs. Total	129.96	1	129.96			
Linear vs. Mean	0.020	1	0.020	1.865E-003	0.9666	
Quadratic vs. Linear	39.91	1	39.91	6.39	0.0394	
Cubic vs. Quadratic	0.22	1	0.22	0.030	0.8677	
Quartic vs. Cubic	25.68	1	25.68	7.19	0.0437	
Fifth vs. Quartic	17.33	1	17.33	133.81	0.0003	Suggested
Sixth vs. Fifth	0.42	1	0.42	12.54	0.0383	Suggested
Residual	0.100	3	0.033			

Table 4: Sequential model sum of squares (Type I).

Source	Sum of Squares	df	Mean Square	F-Value	p-value Prob > F	
Linear	83.56	5	16.71	501.35	0.0001	
Quadratic	43.65	4	10.91	327.38	0.0003	
Cubic	43.43	3	14.48	434.32	0.0002	
Quartic	17.75	2	8.87	266.23	0.0004	
Fifth	0.42	1	0.42	12.54	0.0383	Suggested
Sixth	0.000	0				Suggested
Pure Error	0.100	3	0.033			

Table 5: Lack of fit tests.

Source	Std. Dev.	R-Squared	Adjusted R- Squared	Predicted R- Squared	PRESS	
Linear	3.23	0.0002	-0.1247	-0.6996	142.22	
Quadratic	2.50	0.4772	0.3278	0.0947	75.76	
Cubic	2.69	0.4798	0.2197	-0.2247	102.48	
Quartic	1.89	0.7867	0.6161	-0.7483	146.29	
Fifth	0.36	0.9938	0.9861	0.7412	21.65	Suggested
Sixth	0.18	0.9988	0.9964		+	Suggested

Table 6: Model summary statistics.

may cause process instability in wet anaerobic digestion due to under production and over production of volatile fatty acids which is a key factor in biogas production [18].

Population of cultured bacteria in rumen juice, substrate and digestate

The population of aerobic bacteria and anaerobic bacteria groups inside the rumen juice before and after subjecting it to anaerobic digestion (for two months) in the dark was recorded to be 3.1×10^3 CFU/ml and 4.2×10^2 CFU/ml and 3.4×10^5 CFU/ml and 3.4×10^7 CFU/ml respectively. This result actually shows that the population of aerobic bacteria decreased while the population of anaerobic bacteria increased after the two months. The aerobic bacteria population recorded inside the rumen juice after the two month period may in fact be facultative anaerobes rather than obligate aerobes, which explains why they would have survived the anaerobic digestion process at the time. Aerobic and anaerobic bacterial populations in the substrate were recorded at 3.2×10^5 CFU/g and 3.0×10^3 CFU/g respectively while the populations of aerobic and anaerobic bacteria groups in composite sample of the digestate were recorded at 3.5×10^3 CFU/ml and 5.6×10^7 CFU/ml respectively. Again, the aerobic bacteria population recorded in the composite digestate after the digestion process may in fact be facultative

Source	Sum of Squares	df	Mean Square	F-Value	p-value Prob > F	
Model	83.58	6	13.93	417.89	0.0002	significant
A-Substrate	10.64	1	10.64	319.18	0.0004	
A^2	0.45	1	0.45	13.48	0.0350	
A^3	13.42	1	13.42	402.55	0.0003	
A^4	0.61	1	0.61	18.37	0.0233	
A^5	13.35	1	13.35	400.56	0.0003	
A^6	0.42	1	0.42	12.54	0.0383	
Pure Error	0.100	3	0.033			
Cor Total	83.68	9				

Table 7: ANOVA for response surface sixth model (partial sum of squares - Type III).

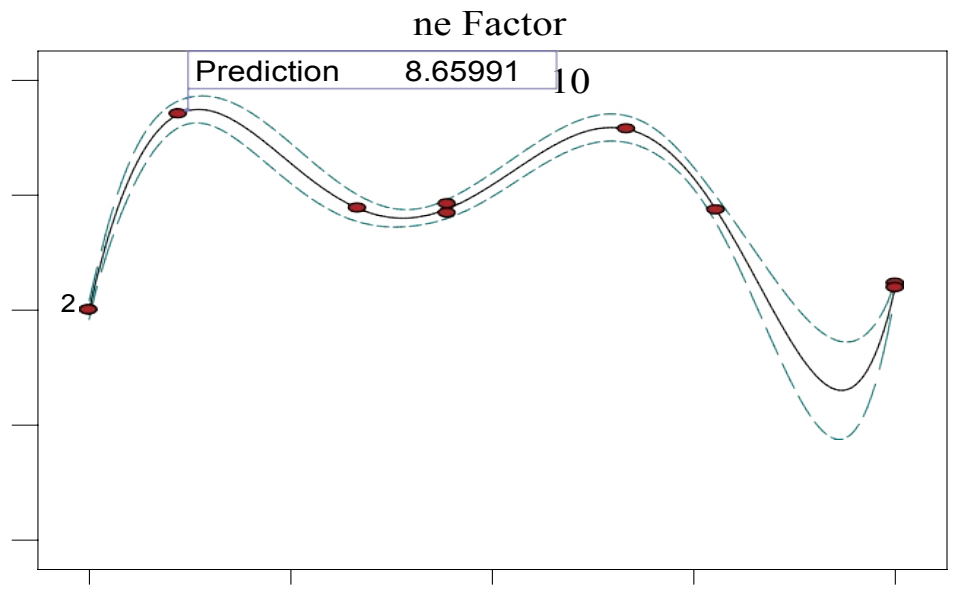

Figure 14: A plot of the response.

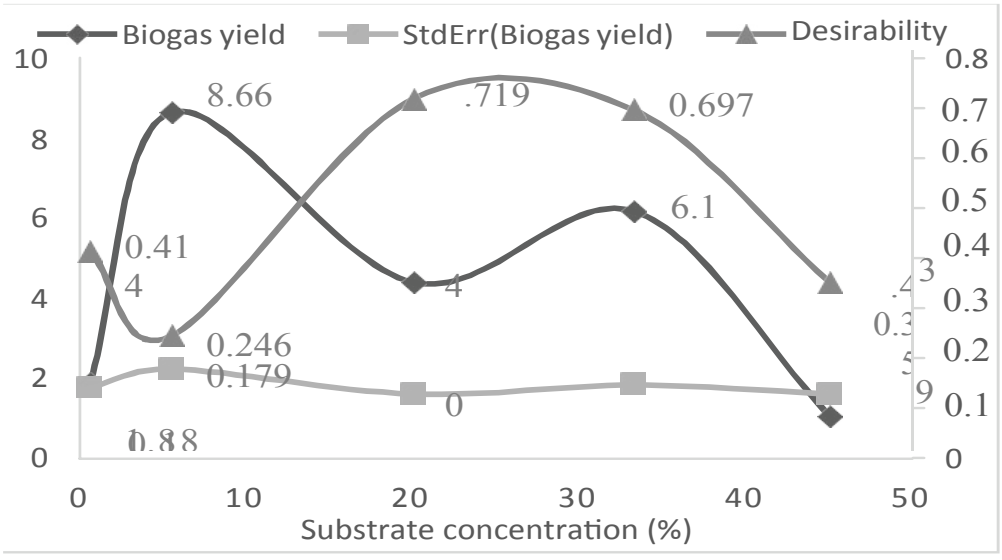

Figure 15: Solutions derived from optimization analysis.

anaerobes rather than obligate aerobes. The distribution of bacteria isolated from the rumen juice, substrate (OFMSW) and composite digestate have been presented in Figure 16. In general, we isolated and identified bacteria species belonging to genera such as Bacillus, Bacteroides, Clostridium, Enterobacter, Escherichia, Lactobacillus, Micrococcus, Morganella, Propionibacterium, Pseudomonas, Providencia, Ruminococcus, Staphylococcus and Streptococcus (Tables 8 to 11). Most of the bacteria genera isolated from the composite digestate were very much similar to most of the genera we isolated from the rumen juice and substrate respectively (Figure 16). This suggested that these bacteria groups may have been involved in the digestion process at some point.

Conclusion

In this study, we subjected a range of concentration (0% - 45%) of pre-treated OFMSW to One- Factor Response Surface design (DX version 9.0) as well as lab-scale anaerobic digestion under ambient condition in order to determine the optimum substrate concentration

Biochemical Tests	B8	B9	B10	B11	B12	B13
Gram stain	+	+	+	+	+	+
Shape	Rod	Rod	Rod	Rod	Rod	Rod
Arrangement	Chain	Chain	Chain	Single	Single	Single
Spore	+	+	+	+	+	+
Acid fast	-	-	-	-	-	-
Motility	+	+	+	+	+	-
O_2 requirement	FA	FA	FA	OAN	OAN	OAN
Oxidase	-	+	+	-	-	-
Coagulase	-	+	-	-	-	-
Citrate	+	-	+	-	-	-
Catalase	+	+	+	+	-	-
Indole	-	-	-	-	-	-
Urease	+	+	-	-	-	-
H_2S Production	-	-	-	-	-	-
Nitrate red.	+	+	+	-	+	-
Voges Proskauer	+	+	+	-	-	-
Ornithine	-	-	-	-	-	-
decarboxylase D-glucose	+/+	+/+	+/+	+/+	+/+	+/+
D-mannitol	-	+/+	-	-	-	+/+
D-sucrose	+/+	+/+	+/+	+/+	-	+/+
Lactose	-	-	-	+/+	-	+/+
D-maltose	+/+	+/+	+/+	+/+	+/+	+/+
D-xylose	+/+	-	+/+	+/+	+/+	+/+
L-arabinose	-	+/+	-	+/+	-	+/+
Salicin	-	-	-	+/+	+/+	+/+
Cellulose	+/-	+/-	+/-	+/+	-	+/+
Starch	+/-	+/-	+/-	+/-	-	+/-
Gelatin	**Methyl red**	-	-	-	+	+
Esculin	+/-	+/-	+/-	+/-	-	-
Glycerol	-	-	-	-	-	+/+
D-cellobiose	+/+	+/+	+/+	+/+	+/+	+/+
D-mannose	-	+/+	+/+	+/+	+/+	+/+
D-melezitose	-	-	-	-	-	-
D-raffinose	+/+	+/+	-	-	-	+/+
D-sorbitol	-	-	+/+	-	-	+/+
L-rhamnose	-	-	+/+	-	-	+/+
D-trehalose	-	-	-	-	+/+	+/+
Probably identify	*Bacillus sp*	*Bacillus sp*	*Bacillus sp*	*Clostridium*	*Clostridium*	*Clostridium*
				sp	*sp*	*sp*

Note: OA=Obligate aerobe, OAN=Obligate anaerobe, FA=Facultative anaerobe, +/+ =Acid and gas production; +/- =Acid production without gas production, - =No fermentation

Table 8: Biochemical characteristics of bacteria isolated during Lab-scale AD study.

Biochemical Tests	B14	B15	B16	B17	B18
Gram stain	-	-	+	+	-
Shape	Rod	Rod	Cocci	Cocci	Rod
Arrangement	Single	Single	Cluster	Chain	Single
Spore	-	-	-	-	-
Acid fast	-	-	-	-	-
Motility	+	+	+	-	+
O2 requirement	FA	FA	FA	FA	FA
Oxidase	-	-	+	+	-
Coagulase	-	-	+	+	-
Citrate	-	+	-	-	+
Catalase	+	+	+	-	+
Indole	+	-	-	-	+
Urease	-	-	+	+	+
H_2S Production	-	-	-	-	-
Nitrate red.	+	+	-	-	+
Methyl red	+	-	-	+	-
Voges Proskauer	-	+	-	+	-
Ornithine decarboxylase	+	+	-	-	+
D-glucose	+/+	+/+	+/+	+/+	+/+
D-mannitol	+/+	+/+	+/-	+/-	-
D-sucrose	+/+	+/+	+/+	+/+	-
Lactose	+/+	+/+	+/+	+/+	-
D-maltose	+/+	+/+	+/+	+/+	+/+
D-xylose	+/+	-	-	-	-
L-arabinose	+/+	+/+	-	+/+	-
Salicin	+/+	+/+	-	-	-
Cellulose	-	-	-	-	-
Starch	-	-	-	+/-	-
Gelatin	-	-	+/-	-	-
Esculin	+/-	-	+/-	+/-	-
Glycerol	+/+	-	-	-	-
D-cellobiose	-	+/+	-	-	-
D-mannose	+/+	-	-	+/+	+/+
D-melezitose	-	-	-	-	-
D-raffinose	-	+/+	-	+/+	-
D-sorbitol	-	-	-	-	-
L-rhamnose	+/+	+/+	-	-	-
D-trehalose	-	-	-	+/+	-
Probably identify	*Escherichia coli*	*Enterobacter sp*	*Staphylococcus sp*	*Streptococcus sp*	*Morganella sp*

Note: OA=Obligate aerobe, OAN=Obligate anaerobe, FA=Facultative anaerobe, +/+ =Acid and gas production; +/- =Acid production without gas production, - =No fermentation.

Table 9: Biochemical characteristics of bacteria isolated during Lab-scale AD study.

Biochemical Tests	B19	B20	B21	B22	B23
Gram stain	+	+	+	-	-
Shape	Cocci	Cocci	Rod	Rod	Rod
Arrangement	Pair	Pair	Pair	Single	Single
Spore	-	-	-	-	-
Acid fast	-	-	-	-	-
Motility	-	-	-	+	+
O_2 requirement	OAN	OAN	OAN	OAN	FA
Oxidase	-	-	-	-	-
Coagulase	-	-	-	-	-
Citrate	-	-	-	-	+
Catalase	-	-	-	-	+
Indole	-	-	-	-	+
Urease	+	-	-	+	+

H_2S Production	+	-	-	+	-
Nitrate red.	-	-	+	-	+
Methyl red	-	-	-	+	+
Voges Proskauer	-	-	-	-	-
Ornithine decarboxylase	-	-	-	-	-
D-glucose	+/+	+/+	+/+	+/+	+/+
D-mannitol	+/+	-	-	+/+	+/+
D-sucrose	+/+	+/+	+/+	+/+	-
Lactose	+/+	+/+	-	+/+	-
D-maltose	+/+	+/+	+/+	+/+	-
D-xylose	+/+	+/+	-	+/+	-
L-arabinose	+/+	+/+	+/+	+/+	+/+
Salicin	+/+	+/+	-	+/+	-
Cellulose	+/+	+/+	-	-	-
Starch	-	+/-	+/-	+/+	-
Gelatin	-	-	+/-	-	-
Esculin	+/-	+/-	-	+/-	-
Glycerol	-	-	+/+	-	-
D-cellobiose	+/+	+/+	-	-	-
D-mannose	+/+	+/+	-	+/+	+/+
D-melezitose	+/+	-	-	-	-
D-raffinose	+/+	+/+	-	+/+	-
D-sorbitol	+/=	+/+	-	-	-
L-rhamnose	+/+	+/+	-	+/+	-
D-trehalose	+/+	+/+	-	-	-
Probably identify	*Ruminococcus sp*	*Ruminococcus sp*	*Propionibacterium sp*	*Bacteroides sp*	*Providencia sp*

Note: OA=Obligate aerobe, OAN=Obligate anaerobe, FA=Facultative anaerobe, +/+ =Acid and gas production; +/- =Acid production without gas production, - =No fermentation.

Table 10: Biochemical characteristics of bacteria isolated during Lab-scale AD study.

Biochemical Tests	B24	B25	B26	B27	B28	B29
Gram stain	+	+	+	+	+	+
Shape	Cocci	Cocci	Rod	Rod	Rod	Rod
Arrangement	Cluster	Cluster	Single	Single	Chain	Chain
Spore	-	-	-	-	-	-
Acid fast	-	-	-	-	-	-
Motility	-	-	+	+	+	+
O_2 requirement	OA	OA	FA	OA	OAN	OAN
Oxidase	+	+	+	+	-	-
Coagulase	-	-	+	-	-	-
Citrate	-	+	+	+	+	-
Catalase	+	+	+	+	-	-
Indole	-	-	-	-	-	-
Urease	-	+	+	+	+	-
H_2S Production	-	-	-	-	-	+
Nitrate red.	+	-	+	-	-	-
Methyl red	+	-	-	-	-	-
Voges Proskauer	+	+	-	-	-	-
Ornithine decarboxylase	-	-	-	-	-	-
D-glucose	+/-	+/-	-	+/-	+/+	+/+
D-mannitol	+/-	-	+/-	+/+	+/+	+/+
D-sucrose	+/-	-	-	-	+/+	+/+
Lactose	-	-	-	+/+	+/+	+/+
D-maltose	+/-	-	-	+/+	+/+	+/+
D-xylose	-	-	-	-	+/+	-
L-arabinose	-	-	-	-	+/+	+/+
Salicin	-	-	-	-	+/+	-
Cellulose	-	-	-	-	-	-
Starch	-	-	-	-	-	-

Gelatin	+/-	+/-	+/-	+/-	-	-
Esculin	-	-	+/-	-	-	-
Glycerol	-	-	+/+	-	-	-
D-cellobiose	-	-	-	-	+/+	+/+
D-mannose	-	-	-	-	-	+/+
D-melezitose	-	-	-	-	-	-
D-raffinose	-	-	-	-	-	-
D-sorbitol	-	-	-	-	+/+	-
L-rhamnose	-	-	-	-	-	+/+
D-trehalose	-	-	-	-	-	-
Probably identify	*Micrococcus sp*	*Micrococcus sp*	*Pseudomonas sp*	*Pseudomonas sp*	*Lactobacillus sp*	*Lactobacillus sp*

Note: OA=Obligate aerobe, OAN=Obligate anaerobe, FA=Facultative anaerobe, +/+ =Acid and gas production; +/- =Acid production without gas production, - =No fermentation

Table 11: Biochemical characteristics of bacteria isolated during Lab-scale AD study.

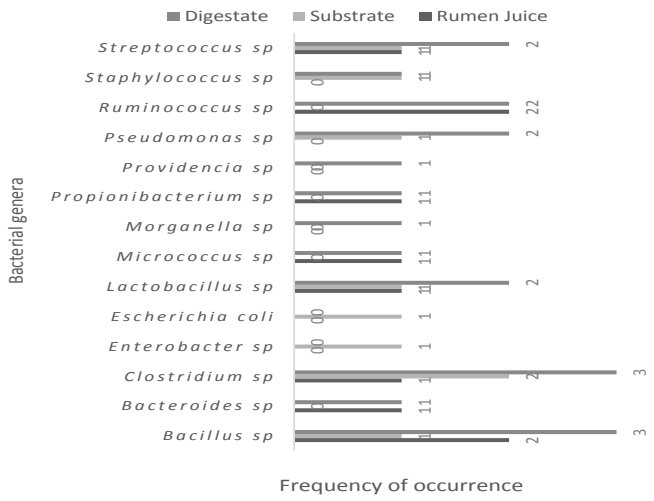

Figure 16: Distribution of bacterial isolated from rumen juice, substrate and digestate respectively.

(%) that will maximize biogas yield under wet process. Result revealed that the optimum substrate concentration which maximized biogas yield was approximately 5.52% (for the wet process). Therefore, in our next study, we will subject this substrate concentration (5.52%) to pilot scale wet anaerobic digestion of organic fraction of municipal solid waste (OFMSW) in 250 L- capacity anaerobic digesters in order to study microbial ecology behind the anaerobic digestion process under ambient condition.

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