American Journal of
Agriculture
(AJA)

BIODIGESTION EFFECTS OF COW DUNG, POULTRY DROPPINGS AND MAIZE COBS ON MICROBIOLOGICAL AND PHYSICO-CHEMICAL PROPERTIES OF THE EFFLUENTS

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AJP

American Journal of Agriculture

ISSN 2520-4678 (Online)
Vol.2, Issue 1 No.1, pp 1 - 16, 2019

www.ajpojournals.org

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ABSTRACT

Purpose: To assess its effects on microbial community, biogas yield and some physico-chemical properties of the effluents.

M**ethodology:** Triplicate slurries of each of the biomass were separately loaded into locally constructed batch-reactor systems, under strict anaerobic condition and kept for eight(8) week retention period. Separate treatment fractions were subjected to standard methods to determine their microbial contents before and during anaerobic digestion (AD). Weekly variations in temperature and weight were followed during the retention period.

Findings: The microbial isolates included 7fungal species, Six (6) non-methanogens, four (4) methanogens and two (2) yeasts. Only *Chaetomium thermophile*, *Aspergillus fumigates* and *Aspergillus nidulans* were isolated at the 5th WOD. The methanogens were predominantly present throughout the digestion period, with increased frequency of occurrence ranging from 50-100%. There was a general % reduction in total viable counts for all microbial isolates, except for the methanogens, with %increase ranging from 83.48% -205.42%. Treatments E(2961.0ml) and B(1713.2ml) had the highest and lowest significant($p < 0.05$) cumulative biogas production, with the co-substrates yielding more than the mono-substrates. All treatments showed progressive temperature rise and average weight loss, which suddenly dropped after the 6th and 4th WOD respectively, with the average weight loss ranging from 23.7±1.9 to 34.3±4.6.

Contribution to theory, practice and policy: There was a strong positive correlation between gas production and weight loss as well as with temperature variation. This initiative engendered alternative energy source, agro-wastes management, while ensuring sustainable environmental rejuvenation.

Key words: *Bio digestion effects, cow dung, poultry droppings, maize cobs, physico-chemical properties, effluents.*

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INTRODUCTION

Co-digestion is the simultaneous digestion of more than one type of waste in the same unit (Okewale, Omoruwou, & Anih, 2018). Advantages include better digestibility, enhanced biogas production/methane yield arising from availability of additional nutrients, as well as a more efficient utilization of equipment and cost sharing (Parawira & Mshandete, 2009). Esposito *et al.* (2012), highlighted other benefits to include: dilution of the potential toxic compounds eventually present in any of the co-substrates involved; adjustment of the moisture content and pH; supply of the necessary buffer capacity to the mixture; increase of the biodegradable material content and widening the range of bacterial strains taking part in the process. This phenomenon influenced by factors such as pH, temperature, C:N ratio, retention time, etc. (Bolzonella, Battistoni, Susini, & Cecchi, 2006). According to Matheri, Belaid, Seodigeng & Ngila (2016), co-digestion of manures and other substrates increase carbon to nitrogen (C/N) ratio and concentration of micro and macronutrients that leads to increase in biogas production.

The hydrolysis which is the first of the four anaerobic digestion steps, involves the degradation of large organic polymers such as fats, proteins and carbohydrates into fatty acids, amino acids and simple sugar respectively. The two acidic stages are the Acidogenesis and Acetogenesis lead to the formation of acetate. These are followed by the methane-forming (methanogenesis) stage.

The biogas technology not only provides environmentally friendly, cost effective (production) and a promising renewable alternative energy source, but also reduces disposable volume of materials and preventing soil and groundwater pollution (Esposito *et al.*, 2012). Furthermore, the semi-solid by-product called digestate produced during the process, is nutrient-rich, and can be used in agriculture directly as bio-fertilizer (Rehl &Mu¨ller 2011).

Since biogas production is associated with microorganisms playing a paramount role in the process (Kumar, Mondal, Gaikward, Devotta & Singh, 2004), it becomes imperative to assess the implication of the process on the microbial loads, and biochemical quality of the digestates.

MATERIALS AND METHODS

Triplicate samples from different slurries obtained as a 1.0kg mixture of dried pulverized maize cob, poultry droppings and cow dung (in different ratios) with sterile distilled water (1:3 ratio w/v, Chomini, 2017). The co-substrate mixtures of the agro-wastes were described as follow:-

TA= 0.0g maize cob + 0.0g poultry droppings + 1000.0g cow dung (0:0:1 ratio)

TB = 0.0g maize cob + 1000.0g poultry droppings + 0.0g cow dung(0:1:0 ratio)

TC = 1000.0g maize cob + 0.0g poultry droppings + 0.0g cow dung(1:0:0 ratio)

TD= 0.0g maize cob + 500.0g poultry droppings + 500.0g cow dung(0:1:1 ratio)

TE = 500.0g maize cob + 500.0g poultry droppings + 0.0g cow dung(1:1:0 ratio)

TF = 500.0g maize cob + 0.0g poultry droppings + 500.0g cow dung(1:0:1 ratio)

TG = 333.3gmaize cob + 333.3g poultry droppings + 333.3g cow dung(1:1:1ratio)

Each of the slurries was separately loaded into a 13.6L capacity sterilized digester, with fittings of thermometer, gas delivery pipe and made airtight to ensure anaerobic condition. The twenty one (21) experimental units were arranged in a completely randomized design (CRD) under a uniform condition in an experimental cubical. The digesters were manually agitated regularly for one minute daily to ensure homogenous condition, and kept for an 8-week retention time. (Chomini, Ogbonna, Falemara & Micah, 2015). During this period, weekly biogas production (in dm3/kg) was measured by downward displacement of water by the gas (Ofoefule, Nwankwo &Ibeto, 2010). Before and after retention fractions of samples of the slurries were aseptically drawn for physico-microbiological investigation.

Microbiological Screening of Substrates

Ten grams (10g) of each of the substrates before and during the digestion were mixed with 90mls of sterile distilled water in 250mls Erlenmeyer flask. After standing for 10minutes, following thorough agitation, 1.0ml aliquots of ten-fold serial dilutions of 10^{-4} and 10^{-5} were plated on nutrient agar (NA) fortified with 50µgml^{-1} Nystatin against fungal growth and incubated for 24 – 48 hours at 35ºC. Bacterial colonies were expressed in cfu/g. Aliquots of diluents of each of the substrates were plated in triplicates on Sabouraud's dextrose agar (SDA), fortified with 100mg/ml streptomycin and 15mg/ml of penicillin against bacterial growth and incubated for 72 to 96 hours. Fungal colonies were expressed in cfu/g. Methods of Ogundero (1981) and Hunter-Cevera, Fonda, and Belt (1986) were employed for isolation and characterization of fungi. For methanogens, selective methanogenic bacteria media were used for the isolation, by incubation anaerobically at 37^0C for 24-48h, under 90% nitrogen (N_2) and 10% CO_2 using gas generating kit (Oxoid, BR 0038B)(Balch *et al*., 1979). All microbial colonies formed were sub-cultured and identified using cultural and biochemical characterization. The morphological examinations of the isolates were determined bythe standard procedure of gram-stain and endospore stain (Teo &Teoh, 2011; Bolarinwa & Ugoji, 2010; Eze & Agbo, 2010).

Determination of Change in Weight and Temperature (g) during Anaerobic Digestion

This was done by determining the initial average weight (g) of each of the three digesters per treatment immediately after loading, using weighing balance. Subsequent change in weight was measured weekly for 8 weeks, as a difference between successive average weight and the initial average weight for all treatments (Franke-Whittle,, Confalonieri, Insam, Schlegelmilch, & Körner, 2014). The initial average temperature (^{O}C) of each of the triplicate digesters per treatment was taken from the mercury in glass thermometer, immediately after loading. Subsequent variation in temperature was measured weekly for 8 weeks for all treatments.

Data Analysis

Data obtained on, biogas yield, microbiological and physical properties were subjected to analysis of variance using SPSS version 18.0 and significant means were separated using Least Significant Difference (LSD).

RESULTS AND DISCUSSION

Total Viable Counts (TVC)

The microbial isolates from the experimental substrates prior to microbial digestion included seven (7) species of fungi: *Trichophaea saccata, Aspergillus fumigatus, Aspergillus nidulans, Aspergillus terreus, Humicola insolens, Chaetomium. Thermophile* and *Talaromyces thermophilus*. The bacteria species were six (6) non-methanogens (*Bacillus subtilis, Klebsiella sp.Escherichia coli, Staphylococcus aureus, Streptococcus faecaalis and Clostridum thermocellum*) and four (4) methanogens (*Methanobacterium formicicum, Methanococcus igneus, Methanothermus fervidus, Methanothrix thermophile*). The two (2) yeasts isolates were *Candida albicans* and *Saccharomyces cerevisiae* (Table 1). There were reductions in total viable counts (TVC) of all microorganisms, except the methanogenic isolates during and after the retention (Table 2). According to St-Pierre and Wright (2013), wide varieties of microorganisms have been reported to colonize agricultural wastes and the soil. Cow dung and poultry droppings were observed to have higher TVC for bacteria and fungi than yeasts before anaerobic digestion (AD) (Table 3 and 4). This agrees with Alfa, Adie, Igboro, Oranusi, Dahunsi & Akali (2014),reported higher total viable counts for fungi and bacteria than with water hyacinth before AD. The diversity of fungal and bacterial isolates obtained from the substrates were similar to those screened by Oyewole (2010) and Khalid and Naz (2013), who reported various isolates of methanogens from different organic wastes before AD. The reduction in non-methanogenic isolates during and after the retention (Table 2), had been attributed to reduction in pH of the digesting media within the first 7 days (Alfa *et al*., 2014), accounting for reduction pathogen counts. Chen, Cheng, and Creamer (2008), stated that increased ammonia and ammonium ions and presence of heavy metals like chromium, iron, cobalt, copper, zinc, cadmium, and nickel, manganese, lead, mercury, molybdenum, might be repressive, antagonistic and lethal to the microbes at certain concentrations. Co-substrates provide microbial consortium with different affinity and specific nutrient requirements (Asikong, Udensi, Epoke, Eja, & Antai,2014), selective inhibition of specific pathways by heavy metals, leading to stratification of the community structurally and functionally (Fulladosa, Murat, Martínez,.& Villaescusa, 2005a; Fulladosa, Murat, & Villaescusa, 2005b) as well as disruption of some specific microbial pathways, consequently decline in number and diversity of organisms relying on those pathways.

The frequency of occurrence of the microbial isolates ranged from 28.57%-100%(fungi), 28.57%-100%(non-methanogenic bacteria), 28.57%-42.86%(methanogenic bacteria) and 57.14%-71.43% (yeasts). Most of the fungal species were isolated within up to the 4th week of digestion (WOD). At the 5th WOD, only *Chaetomium thermophile, Aspergillus fumigates* and *Aspergillus nidulans* were isolated. From the 6th to the 8th WOD, no fungal isolates was found in the digesting media (Table 2). Similarly, all the non-methanogenic bacteria were not found beyond the 3rd WOD,

except *Clostridum thermocellum* which was screened up to the 8th WOD. There was no yeast isolate obtained from the 1st to the 8th WOD. However, the methanogens were predominantly present throughout the digestion period, with increased frequency of occurrence ranging from 50-100%(Table 2). Kuang (2002), reported *Clostridium* and *Klebsiella* among the predominant fermentative isolates throughout the digesting period of different organic biomass. There was a general % reduction in total viable counts for all microbial isolates from the digesting media, except for the methanogens with 83.48%, 115.28%, 145.24%, 163.68%, 184.71%, 193.19% and 205.42% as %increase from treatments B, A, C, D, M, E, F respectively. This corroborated the findings of Bolarinwa and Ugoji (2010), who reported a general reduction in total viable counts of all microbial isolates from all the different digesting media.

Biogas Yield, Temperature and Weight Variations during Anaerobic Digestion

All treatments showed a progressive increase in biogas yield in the first six weeks of digestion, followed by a sharp drop up till the end of the process. The average cumulative gas production was in the order of treatment D(2961.0ml) > E(2481.3ml) > F(2442.3) > G(2200.7ml) > B(2197.9ml) > A(2079.0ml) > C(1713.2ml). All the co-substrates had higher yields than the mono-substrates (Table 5). Analysis of variance (ANOVA) indicated significant difference ($p<0.05$) in biogas yield due to substrate types and mixing ratio. The increase in biogas production with retention time within the first 6thWOD, agreed with the finding of Babaee, Shayegan and Roshani (2013), which who attributed this to substrate composition, microbial content and temperature, while describing the point of decline as the break point. The nature of the substrate to a large extent affects the biogas yield. Poultry droppings and cow dung recorded higher yields due to their relatively higher nitrogen content as posited by Kassuwi, Mshandete and Kivaisi (2012). The higher yields obtained from the co-substrates over the single corroborated the findings of Ofosu and Aklaku, (2010), due to higher process stability. Esposito *et al*. (2012), indicated that co-digestion provides optimization of nutrient balance due to buffering capacity and interesting synergistic effect (Wu, Yao, Zhu, Miiler, 2010), while making metals more concentrated in dry sludge as compared to mono-substrate process (Lebiocka, Montusiewicz & Depta, 2016).

The rise in temperature followed the same pattern of gas production, whereby a sudden drop between the 6th and the 8th week was preceded by an initial rise (Table 6). Treatments C(44.1 ± 0.3^0C) and G(41.0 ± 0.5^0C) recorded the highest and lowest average temperature at peak of the digestion time, while E(29.8 ± 0.3^0C) and C(27.6 ± 0.2^0C) were at the terminal of the process. The decline in temperature negatively affected the volume of gas production (Figure 1). This was similar to the report of Chae, Jang, Kim and Yim (2008), indicating different biogas composition at different digestion temperature, with methane contents in the biogas linearly related to temperature change, where 65.3%, 64.0% and 62.0% at were produced at 35°C, 30°C and 25°C, respectively. Jafari, Afazeli, Rafiee, Nosrati, and Almasi (2014), in their finding posited an optimal condition of temperature (36-40^{0}C), stirring (one minute daily) and mixing ratios of 1:2 and 1:1 of cow dung and poultry droppings as best for biogas production. Temperature increase is known to lead to an increase in the maximum specific growth and substrate utilization, and much faster biochemical reaction rates (Gao, Leung, Qin & Liao 2011) and increase in biogas production from cow dung, pig and poultry manures (Prasad, 2012). Gao *et al.* (2011), observed that a sudden increase or

decrease in temperature by 10ºC leads to temperature shocks at 45ºC, prompting death rate exceeding growth rate and consequently serious drop in treatment efficiency, which could take about 16 days to recover before methane production resumes. They also maintained that the phenomenon decreases the chemical oxygen demand removal efficiency (from 80.6% to 53.3%), and also affects the diversity and species richness, impacting negatively on the microbial community structure (Choorit & Wisarnwan, 2007).

The weekly variation in substrates weight loss due to anaerobic digestion followed the same trend proportionately as with temperature. There was a strong positive correlation between gas production and weight loss as well as with temperature variation (Figure 2). All treatments recorded highest reduction in average weight at the 4th week of digestion (WOD), with treatments E(118.5±2.1) and B(86.8±3.8) as the highest and lowest values respectively. However at 8(WOD), the average weight loss ranged from 23.7±1.9 to 34.3±4.6.The progressive increase in weight loss recorded from week 1 to 4 agrees with the findings of Li *et al*. (2011), who related the reduction of organic wastes of effluents to their biodegradability efficiency, terms of total solid, volatile solid, chemical oxygen demand and total organic carbon reductions. Schafer *et al*. (2006), related the residual weights of the effluents as the difference between fresh weight and weight of digestates removed. Jha, Li, Zhang, Ban, and Jin (2013), described the efficiency of degradation as a function of biological conversion of the substrates due volatile solid or chemical oxygen demand removal with simultaneous production of biogas leading to reduction of organic waste. Consequently, the differential between the initial and final weight values reflects the level of removal, as the bioconversion efficiency index. Volatile solids and chemical oxygen demand removal efficiencies of organic waste can be enhance under thermophilic condition than mesophilic temperature (Jha *et al* ., 2013). The pattern of correlation between average volume of gas produced and average weight loss suggestively reflect close link between material utilization and biogas production. The correlation varies with treatments. Bhattacharya and Mishra (2005) and Jha, Narsaiah, Sharma, Singh, Bansal, and Kumar (2010a), reported close relationships between biogas yield and total solid, volatile solid, chemical oxygen demand and total organic carbon removal. El-Mashad and Zhang, (2010), affirmed that biogas production increase with an increase in chemical oxygen demand removal and volatile solid reduction.

CONCLUSION

The study has revealed reduction in total viable counts and frequencies of occurrence of non-methanogenic microorganisms and increase in the methanogenic isolates. Average cumulative biogas production, in the order of treatment E(2961.0ml) >F(2481.3ml) > D(2442.3) > G(2200.7ml) > C(2197.9ml) > A(2079.0ml) > B(1713.2ml). All the co-substrates had higher yield values than the mono-substrates. There was a strong positive correlation between gas production and weight loss as well as with temperature variation.

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American Journal of Agriculture
ISSN 2520-4678 (Online)
Vol.2, Issue 1 No.1, pp 1 - 16, 2019
www.ajpojournals.org

Table 1: Microbial Isolates before Anaerobic Digestion of Substrates

Microbial Isolates	A	B	C	D	E	F	G	Total	% frequency of occurrence
FUNGI									
Trichophaea saccata	+	-	-	-	-	-	+	2	28.57
Aspergillus fumigatus	+	+	+	+	+	+	+	7	100.0
Aspergillus nidulans	+	+	+	+	+	+	+	7	100.0
Aspergillus terreus	+	+	+	+	+	+	+	7	100.0
Humicola insolens	+	+	+	+	+	+	+	7	100.0
Chaetomiu. thermophile	+	-	-	+	+	-	+	4	57.14
Talaromyces thermophilus	+	+	+	+	+	+	+	7	100.0
Total	7	5	5	6	6	5	7	41	
YEAST									
Candida albicans	-	+	+	+	+	+	-	5	71.43
Saccharomyces cerevisiae	+	-	-	+	+	-	+	4	57.14
Total	1	1	1	2	2	1	1	09	
BACTERIA									
Bacillus subtilis	+	-	+	+	+	+	-	5	71.43
Klebsiella	-	+	-	-	-	-	+	2	28.57
Escherichia coli	+	+	-	-	+	-	+	4	57.14
Staphylococcus aureus	-	-	+	-	+	+	+	4	57.14
Streptococcus faecaalis	-	-	-	+	-	+	-	2	28.57
Clostridum thermocellum	+	+	+	+	+	+	+	7	100.0
Methanobacterium formicicum	+	-	+	-	-	-	-	2	28.57
Methanococcus igneus	+	-	+	-	-	-	+	3	42.86
Methanothermus fervidus	+	-	+	-	-	-	+	3	42.86
Methanothrix thermophile	+	-	+	+	-	-	-	3	42.86
Total	7	3	7	4	4	4	6	35	
Grand Total	15	9	13	12	12	10	14	85	

American Journal of Agriculture
ISSN 2520-4678 (Online)
Vol.2, Issue 1 No.1, pp 1 - 16, 2019
www.ajpojournals.org

Table2: Microbial Isolates During Anaerobic Digestion of Substrates

Microbial Isolates	Week 1	2	3	4	5	6	7	8	Total	%Frequency of occurrence
Fungi										
Chaetomium thermophile	+	+	+	+	+	-	-	-	5	62.5
Talaromyces. thermophilus	+	+	+	+	-	-	-	-	4	50.0
Trichophaea saccata	+	+	-	-	-	-	-	-	2	25.0
Aspergillus fumigatus	+	+	+	+	+	-	-	-	5	62.5
Aspergillusn nidulans	+	+	+	+	+	-	-	-	5	62.5
Aspergillus terreus	+	+	+	+	-	-	-	-	4	50.0
Humicolainsolens	+	+	+	+	-	-	-	-	4	50.0
Total	**07**	**07**	**06**	**06**	**03**	**00**	**00**	**00**	29	
Yeasts										
Candida albicans	-	-	-	-	-	-	-	-	0	0.0
Saccharomyces cerevisiae	-	-	-	-	-	-	-	-	0	0.0
Total	**00**	**00**	**00**	**00**	**00**	**00**	**00**	**00**	0	
Bacteria										
Bacillus subtilis	+	+	+	-	-	-	-	-	3	37.5
Klebsiella sp	+	+	-	-	-	-	-	-	2	25.0
Escherichia coli	+	+	+	-	-	-	-	-	3	37.5
Staphylococcus aureus	+	+	+	-	-	-	-	-	3	37.5
Streptococcus faecalis	+	+	-	-	-	-	-	-	2	25.0
Clostridum thermocellum	+	+	+	+	+	+	+	+	8	100.0
Methanobacterium formicicum	+	-	-	-	+	+	+	+	5	50.0
Methanococcus igneus	+	+	+	+	+	+	+	+	8	100.0
Methanothermus fervidus	+	-	-	-	+	+	+	+	5	50.0
Methanothrix thermophile	+	+	+	+	+	+	+	+	8	100.0
Total	**10**	**08**	**06**	**03**	**05**	**05**	**05**	**05**	**47**	
Grand Total	**17**	**15**	**12**	**09**	**08**	**05**	**05**	**05**	**76**	

American Journal of Agriculture
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Table 3: Microbial Counts (cfu/ml) of the Experimental Substrates before and after the Anaerobic Digestion (Logarithmic transformed data Log10)

**Tmt	TFC BAD	TFC AAD	%Effect of AD	TCC BAD	TCC AAD	%Effect of AD
**A	5.30	3.08	-41.89	3.48	0.00	-100.0
B	4.00	2.79	-30.25	3.00	0.00	-100.0
C	6.04	4.40	-27.15	4.00	0.00	-100.0
D	5.00	3.36	-32.80	3.60	0.00	-100.0
E	5.90	3.79	-35.76	3.70	0.00	-100.0
F	5.70	3.45	-39.47	3.60	0.00	-100.0
G	5.85	3.72	-36.41	4.15	0.00	-100.0

TFC= Total FungalCount, TCC= Total Coliform Count, BAD = Before Anaerobic Digestion; AAD = After Anaerobic Digestion

Table 4: Microbial Counts (cfu/ml) of the Experimental Substrates before and after the Anaerobic Digestion (Logarithmic transformed data Log10)

**Tmt	*TBC BAD	TBC AAD	%Effect of AD	TMC BAD	TMC AAD	%Effect of AD
**A	4.60	2.36	-48.70	3.01	6.48	115.28
B	4.30	2.04	-52,56	0.88	1.61	84.09
C	5.48	3.11	-43.25	2.51	6.11	143.43
D	4.90	3.72	-24.08	2.01	5.60	163.68
E	5.15	3.04	-40.97	2.03	6.20	205.42
F	5.11	3.04	-40.51	1.80	5.29	193.89
M	5.20	3.80	-26.92	1.83	5.20	184.15

*TBC= Total Bacterial(non- methanogenic) Count;TMC = Total methanogenicbacterial CountBAD =Before Anaerobic Digestion; AAD = After Anaerobic Digestion

American Journal of Agriculture
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Table 5: Mean Gas Production (ml/wk) During Eight Weeks of Anaerobic Digestion

	Weeks								
Tmt	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Total**
A	66.7	110.0	177.3	320.7	358.0	393.0	381.3	272.0	2079.0
B	93.3	150.7	262.7	316.3	382.3	423.3	385.0	184.3	2197.9
C	43.3	78.3	134.3	287.3	321.3	348.7	303.3	196.7	1713.2
D	98.3	176.7	280.3	345.7	447.3	621.0	562.0	429.7	2961.0
E	63.0	113.0	240.0	309.7	462.3	512.0	418.0	363.3	2481.3
F	76.7	108.0	188.0	328.3	421.7	519.3	437.3	363.0	2442.3
G	83.0	114.7	196.0	328.3	426.0	525.7	398.7	128.3	2200.7
∑	946.0	1542.7	2710.9	3990.0	5093.6	6093.0	5082.6	3603.6	**29062.5**

Tmt = treatment

Table 6: Temperature Variation of Samples during Eight Weeks of Anaerobic Digestion

	Week							
Tmt	1	2	3	4	5	6	7	8
A	29.8±0.3	36.6±1.5	40.3±1.9	42.7±0.1	43.2±0.4	43.5±0.3	35.7±0.6	28.5±0.3
B	30.3±0.1	38.9±0.8	41.5±1.1	42.8±0.4	43.5±0.3	44.1±0.3	36.4±0.2	27.6±0.2
C	30.0±0.2	33.2±0.9	36.3±0.7	38.2±0.7	43.2±0.9	43.6±0.9	35.6±0.2	28.4±0.2
D	30.4±0.7	35.7±0.4	38.2±0.4	39.0±0.5	42.5±0.3	43.2±0.3	36.6±0.2	29.8±0.3
E	29.4±0.2	35.3±0.6	36.5±0.3	38.5±0.3	41.2±0.7	42.1±0.3	35.2±0.6	28.1±0.4
F	29.7±0.5	35.5±2.8	36.8±0.4	37.9±0.3	38.7±0.1	41.4±0.2	35.4±0.2	28.4±0.2
G	29.8±1.4	32.9±0.5	35.7±0.6	37.7±0.7	39.8±0.4	41.0±0.5	35.7±0.6	28.5±0.3

Tmt = Treatment

Table 7: Average Weight Loss (g/wk) during the Eight Weeks of Anaerobic Digestion

	Week							
Trts	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
A	26.4 ± 3.3^{ab}	37.7 ± 2.7^{b}	83.6 ± 5.5^{c}	109.1 ± 8.0^{d}	61.6 ± 9.1^{c}	63.8 ± 7.7^{d}	34.3 ± 5.5^{b}	28.0 ± 1.6^{a}
B	39.3 ± 1.0^{d}	57.4 ± 1.2^{d}	85.0 ± 1.7^{d}	117.6 ± 2.0^{e}	65.9 ± 2.9^{d}	50.7 ± 0.8^{a}	43.1 ± 4.5^{c}	34.3 ± 4.6^{b}
C	23.1 ± 1.5^{a}	32.1 ± 1.4^{a}	67.3 ± 2.3^{a}	86.8 ± 3.8^{a}	50.2 ± 1.3^{a}	48.3 ± 2.0^{a}	30.4 ± 2.8^{a}	23.7 ± 1.9^{a}
D	39.7 ± 1.1^{d}	58.6 ± 1.4^{d}	85.8 ± 1.5^{e}	118.5 ± 2.1^{e}	64.6 ± 4.2^{d}	66.0 ± 3.9^{e}	37.5 ± 0.9^{b}	27.5 ± 1.0^{a}
E	27.8 ± 1.2^{b}	38.1 ± 1.8^{b}	77.5 ± 11.7^{b}	106.2 ± 2.7^{c}	54.4 ± 5.3^{b}	58.5 ± 4.0^{c}	36.5 ± 4.1^{b}	27.5 ± 1.2^{a}
F	25.7 ± 2.4^{ab}	37.5 ± 2.5^{b}	82.9 ± 5.7^{c}	97.9 ± 1.2^{b}	50.0 ± 1.5^{a}	52.1 ± 1.7^{b}	37.1 ± 0.1^{b}	26.7 ± 2.4^{a}
G	28.5 ± 3.5^{c}	40.0 ± 3.1^{c}	68.9 ± 7.6^{a}	104.6 ± 9.7^{c}	55.7 ± 4.4^{b}	57.9 ± 4.1^{c}	32.4 ± 2.1^{a}	24.4 ± 1.2^{a}

American Journal of Agriculture
ISSN 2520-4678 (Online)
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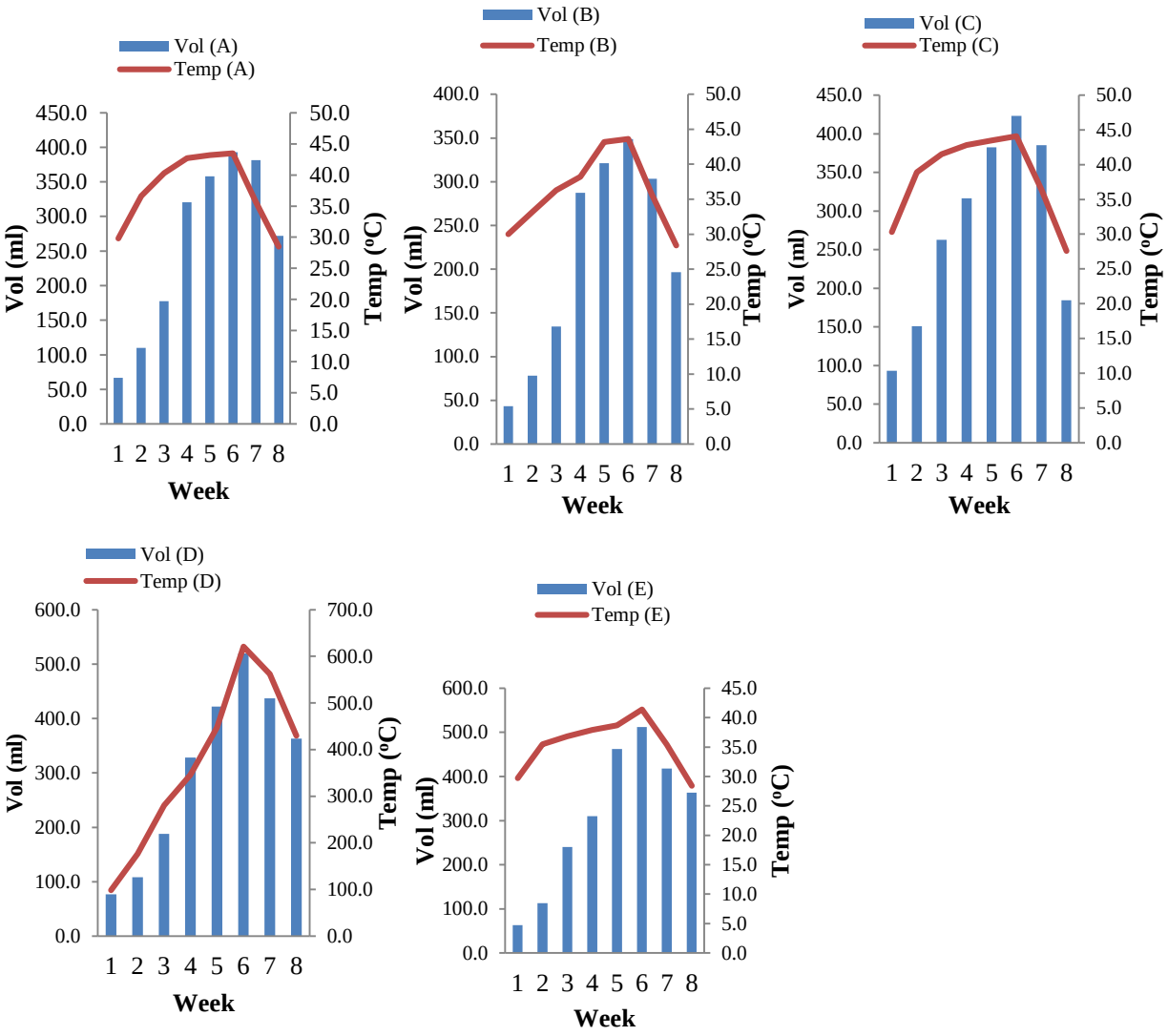
www.ajpojournals.org



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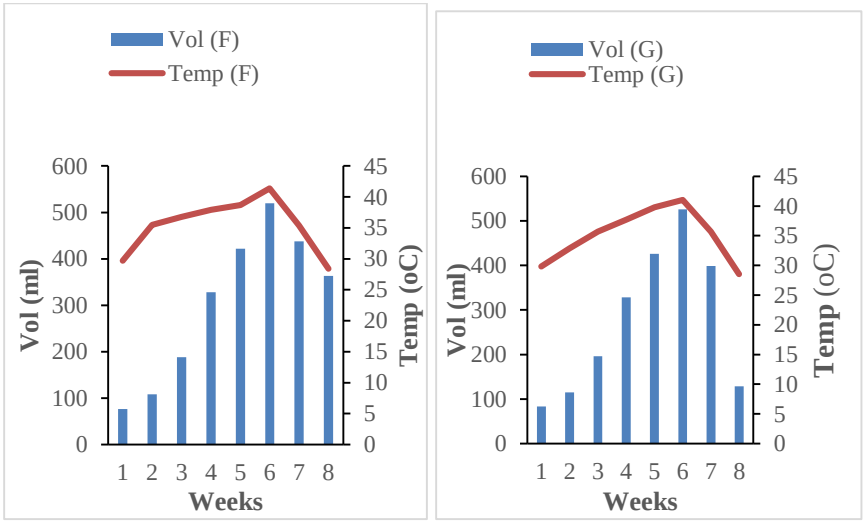

Figure 1: Effects of Temperature Variation on Volume of Biogas Production

American Journal of Agriculture
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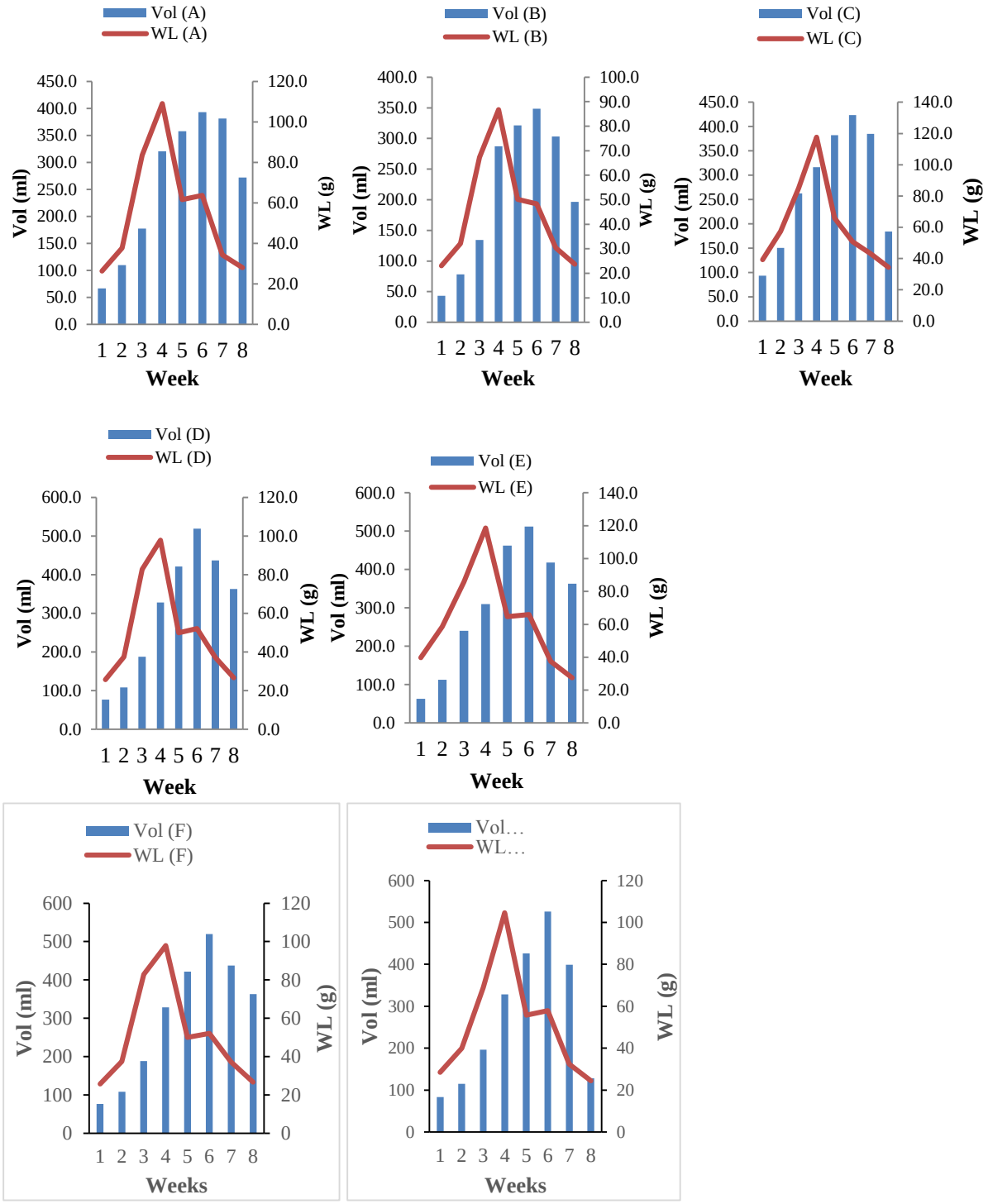

Figure 2: Effects of Weight Loss Variation on Volume of Biogas Production