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Combinatorial Effect of Process Parameters on the Rate of Biogas Production and Rate of Substrate Degradation Following Anaerobic Digestion of Food Waste and Rumen Content

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ABSTRACT: In this study, the combinatorial effect of process parameters on the rate of biogas production and rate of substrate degradation following anaerobic digestion of food waste and rumen content using mixture design (Combined I-optimal) within the Design Expert (version 13) environment. Results showed that the rate of biogas production, rate of bio-methane production and rate of substrate degradation inside the 100 bio-digesters ranged from 0 to 38.04 L/Kg VS, 0 to 23.14 L/Kg VS and 0 to 79.20 %, respectively. The highest rate of biogas production (38.04 L/Kg VS), highest rate of bio-methane production (23.14 L/Kg VS) and highest rate of substrate degradation (79.20 %) were observed in bio-digester 57 at food waste (0.30 kg), rumen content (0.30 kg), water content (0.40 kg), temperature (34.0ºC), pH (9.0), number bio-digester of agitation per day (4 time/day) and retention time (32 days), respectively. The rate of biogas/bio-methane production and rate of substrate degradation can vary, with varying process factors/parameters in anaerobic digestion processes. Bio-digesters with anaerobic co-digestion of the food waste and rumen content appeared to be significantly more productive in terms of biogas/bio-methane production rate and substrate degradation rate compared to the bio-digesters with anaerobic mono-digestion of either food waste or rumen content regardless of the presence other process factors/parameters within the boundaries of this investigation.

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In Nigeria, one of the chief pathways in which food waste is disposed include open dumping and burning in dump sites and sometimes throwing away in landfills. Knowing the rapid biodegradability of food waste in the company of contaminating microbes, open dumping in dump sites or in landfills can be very questionable (Oduniyi *et al*., 2021). Furthermore, the biological degradation of organic matter such as food waste and other types on dumpsites and inside landfills demands an enormous land space in which greenhouse gases such as carbon dioxide, methane, etc., are created with no profitable gained in terms of the energy created by the organic matter in the form of biogas (Babajide and Oloruntoba, 2022). In addition, given the high moisture content of the food waste, open burning necessitates high amounts of energy with no energy recovery in most circumstances (Chinweze *et al*., 2021). Both choices inflict adverse impressions on both man and his environment (Folaranmi *et al*., 2021). This problems have led many countries to place an embargo on disposal options such as open dumping and open burning. Waste-to-energy techniques are habitually considered for the removal of organic wastes materials from the environment because they

support the reduction of environmental impressions and fractional replacement of fossil reserves. One of the most feasible methodologies include anaerobic digestion process, an ecologically responsive technology for transmuting organic wastes into biogas and digestate (Kang *et al*., 2022). Animal rumen content is one of slaughterhouse wastes that is frequently thrown out into the environment. This waste removal arrangement causes ecological irritation, predominantly posing health deathtrap to humans due to its content of millions of microorganisms. Nevertheless, the obtainability of rumen content may be advantageous as an activator in manufacturing biogas via anaerobic degradation, since some of rumen microorganisms are cellulolytic bacteria and methanogenic archaea (Liu *et al*., 2021). Biogas is a type of biofuel that can be produced from anaerobic digestion of organic materials such as agro-food waste, animal waste, municipal waste, plant waste and sewage (Zhang *et al*., 2021). The gas produced has high energy content and can be used for many applications such as heating, cooking, power generation, and lighting as a biofuel (Kumar *et al*., 2022). The conversion of complex organic compounds into methane and carbon dioxide requires different groups of microorganisms and is carried out in a sequence of hydrolysis, acidogenesis, acetogenesis and methanogenesis (Li *et al*., 2021). Biogas generation has been reported to be affected by different factors such as the nature of the substrate to be digested, the nature of the biodigester, temperature, pH, alkalinity, retention time, organic loading rate, carbon/nitrogen (C/N) ratio, nutrient availability, moisture, oxygen, ammonia, volatile fatty acids, microbial inoculum/substrate ratio, particle size, pre-treatment of substrate and inhibitors such as organics, metals and secondary metabolites (Wang *et al*., 2021; Yi *et al*., 2022). The aim of this investigation was to evaluate the combinatorial effect of process parameters such as temperature, pH, number bio-digester agitation/day and solid retention time on the rate of biogas/biomethane production and the rate of substrate degradation following anaerobic digestion of food waste and rumen content.

MATERIALS AND METHODS

Collection of the substrates: Cow rumen content, also used as the main source of microbial inoculum for anaerobic digestion process, was collected from the Dutse Central Abattoir in Dutse, Jigawa State of Nigeria. After collection, the rumen content was immediately transported to the experimental site in an air-tight, non-transparent 60 L-capacity plastic container. Food wastes, which included cooked rice, cooked beans, cooked rice powder meal (Tuwo-Shinkafa), cooked corn powder meal (Tuwo-Masara), boiled yam, wasted bean cake (Akara) and wasted rice and corn cakes (Masa), were collected at their source of generation within Dutse metropolis. After collection, the food wastes were immediately taken to the experimental site, where they were pooled and blended together (with the help of an electric mixer) to form the homogenized food waste that was used for the biogas production process.

Determination of physicochemical properties of the substrates: After collecting the substrates, samples of the rumen content and the homogenized food waste were taken to the laboratory to determine some of their physical and chemical profiles using standard methods. Total dry solids (TS), water content (WC), volatile solids (VS) and ash content (AC) of the substrates were determined using the gravimetric method as described in standard methods (APHA, 2017). Total carbohydrate, crude protein, volatile fatty acid (VFA), ash-free acid detergent lignin (ADL), total nitrogen and total carbon were also determined using standard methods (AOAC International, 2016). Crude lipid was determined by the acid (HCL) hydrolysis method described in standard methods (AOAC International, 2016).

Estimation of microbial populations in the substrates: Bacteria and Archaea populations in the substrates were estimated (by enumeration) based on metabolic specialization and oxygen requirement as described by Ogbonna *et al*. (2018). These included the populations of facultative anaerobic bacteria (FAB), strict anaerobic bacteria (SAB), acetoclastic methanogens (AM) and hydrogenotrophic methanogen (HM).

Design of the experiment: In order to evaluate the combinatorial effect of process parameters such as food waste (0 – 1 kg), rumen content (0 – 1 kg), water content (0 – 1 kg), temperature (28 – 45^{o}C), pH (5 - 9), number digester agitation/day (0 – 6 times/day) and retention time (15 – 40 days) on the rate of biogas and bio-methane production as well as the rate of substrate degradation, food waste and rumen content were subjected to anaerobic mono-digestion and co-digestion using mixture design (Combined I-optimal) within the Design Expert (version 13) environment, which generated a total of 100 experimental runs.

Anaerobic digester specification and set-up: In each of the experimental runs, an air-tight, one-stage, 2L-capacity, plastic anaerobic digester, with useful volume of around 1.9 L and a head space of around 0.1 L was employed for the anaerobic digestion process. The anaerobic digester had a biogas outlet, a feeding inlet and a digestate outlet attached to it (Figure 1). A

biogas cleaning system, which was composed of a carbon dioxide (CO_2), hydrogen sulphide (H_2S) and water (H_2O) vapour removal units (or chambers) was also attached to the biogas outlet of the anaerobic digester (Figure 1). On each of the units which made up the biogas cleaning system, a 300mL-capacity gas measuring syringe was attached to determine the volume of major gaseous components of biogas generated from the anaerobic digester (Figure 1). Furthermore, each of the four units had a volume of 0.3 L-capacity. The first unit contained 0.29 L of saline saturated water, through which total biogas generated in the bio-digester was collected and measured. The second unit contained 0.29 L of potassium hydroxide (KOH) solution and served as the CO_2 remover in the total biogas coming from the first unit. The third unit contained iron (II) oxide in the form of iron filings and served as the H_2S remover in the CO_2-less biogas coming from the second unit. The fourth unit was packed silica gel to remove the H_2O vapour in the H_2S-less CH_4-rich biogas coming from the third unit. At each stage of the biogas cleaning process, the volume of the resultant gas was measured using the gas measuring syringe attached to each of the units (Figure 1).

Fig 1. 2L-Capacity anaerobic digester with biogas cleaning system and gas measuring

Operation of the anaerobic digesters: The temperature of each of the bio-digesters was regularly monitored and regulated using a digital thermostat with a temperature probe that was inserted in the water bath which housed each of the bio-digesters (Sulaiman *et al*., 2020).

The pH of the anaerobic digesters was measure and monitored using a digital pH meter with probe and any change in the pH of the bio-digesters was adjusted using either hydrochloric acid (if the change was alkaline in nature) or potassium hydroxide (if the change was acidic in nature) to maintain its stability for a given experimental run (Montalvo-Rodriguez *et al*., 2022). The anaerobic digesters were agitated manually (by hand-shaking) a number of times per day in order to stimulate mixing of bio-digester contents as well as enhancing biogas production (Zhang *et al*., 2021).

Determination of biogas and methane production rate: The volume of cumulative biogas and its carbon dioxide, hydrogen sulphide, moisture and methane contents were estimated volumetrically using serially connected biogas-separating chambers, each of which had a gas measuring syringe attached, with corresponding biogas separating solutions such as saline saturated solution, potassium hydroxide solution, iron (II) oxide solution and silica gel respectively as described by (Alemawor and Quaye, 2014).

Consequently, the rate of biogas production and rate of methane production in each of the anaerobic digesters were determined using the formulae in Equation 1 and Equation 2 respectively (Fiebig and Menardo, 2015; Singh *et al*., 2012).

$$Bgasr\ (L/kg.VS\} = \frac{VC-Biogas}{MVS} \qquad (1)$$

Where VC-Biogas = Volume of cumulative biogas after digestion and MVS = Mass of volatile solids in the feed before digestion

$$CH_{4r}\ (L/kg.VS\} = \frac{VC-Meth}{MVS} \qquad (2)$$

Where VC-Meth = Volume of cumulative biogas after digestion and MVS = Mass of volatile solids in the feed before digestion

Determination of rate of substrate degradation in the feed: At the end of the anaerobic digestion process, the rate of degradation of the substrates inside the bio-digesters was determined using the formula in Equation 3 as described by Schnurer and Jarvis (2010).

$$SDr\ (\%) = \frac{MBD}{MAD} x\ 100 \qquad (3)$$

Where MBD = Mass of total solids in the feed before digestion and MAD = Mass of total solids in the feed after digestion

RESULTS AND DISCUSSION

Characteristic of the substrates: The result of some physico-chemical and microbiological properties of the substrates which were subjected to anaerobic digestion in the present study is presented in Table 1. Result presented in Table 1 implies that approximately 96.3% of the food waste and 72.6% of the rumen content were biodegradable at the time and should be converted to biogas (Shah *et al*., 2022). The C/N ratio of 29.44 and 22.48 for the food waste and rumen content respectively shows the relationship between the amount of carbon and nitrogen present in the substrates. Values of C/N ratio ranging from 10 to 35 have been reported to have worked well during biogas production from food waste and rumen content, with the optimum said to be between 15 and 30, depending on other conditions (Yue *et al*., 2021). Therefore, the C/N ratio of the substrates used in this study, lie within the working ratio with respect to biogas production. The result in Table 1 also shows the presence of facultative anaerobic bacteria, strict anaerobic bacteria and methanogenic archaea in the rumen content that was employed as the source of microbial inoculum for the anaerobic digestion process. The presence of these groups of organisms in rumen content and their role in biogas production have been reported by several authors (Kim *et al*., 2021; Poghosyan *et al*., 2022). Basically, the facultative anaerobic bacteria and strict anaerobic bacteria play important role in the fermentation processes which produce the substrates for methanogenesis (by methanogenic archaea) to occur. Facultative anaerobic bacteria also help to consume and eliminate any residual oxygen which may have entered the bio-digesters with the substrates, allowing both strict anaerobic bacteria and methanogenic archaea to proliferate and survive within the bio-digester (Chen *et al*., 2021).

Table 1: Characteristic of the substrates

Parameters	Food Waste	Rumen Content
% Wet solid	100	100
% Total (Dry) solid	76.80	78.30
% Water content	23.20	21.70
% Volatile solid	96.30	72.60
% Ash content	3.70	27.40
% Total CHO	63.90	67.34
% Crude protein	20.20	7.20
% Crude lipid	7.78	1.36
% Total VFA	5.90	10.70
% AFD lignin	2.22	13.40
Total carbon (g/kg)	865.51	925.80
Total nitrogen (g/kg)	29.40	41.18
C/N ratio	29.44	22.48
Facultative bacteria count (cfu/g)	6.70×10^5	11.90×10^5
Strict anaerobic bacteria count (cfu/g)	1.14×10^5	17.70×10^5
Acetoclastic methanogens (cfu/g)	0.00	2.90×10^5
Hydrogenotrophic methanogens (cfu/g)	0.00	1.48×10^5

Rate of biogas production: The rate of biogas production between bio-digester 1 and bio-digester 100 ranged from 0 – 38.04 L/Kg VS (Figure 2). The highest rate of biogas production (38.04 L/Kg VS) was recorded in bio-digester 57 followed by bio-digester 58 (36.20 L/Kg VS), bio-digester 46 (34.40 L/Kg VS), bio-digester 73 (32.24 L/Kg VS), bio-digester 10 (31.52 L/Kg VS), bio-digester 60 (30.40 L/Kg VS), bio-digester 45 (27.92 L/Kg VS), bio-digester 50 (27.50 L/Kg VS) and bio-digester 1 (27.10 L/Kg VS). Bio-digester 57, which generated the highest rate of biogas production was operated at a food waste of 0.30 kg, rumen content of 0.30 kg, water content of 0.40 kg, temperature of 34.0ºC, pH of 9.0, bio-digester agitation of 4 times/day and a retention time of 32 days.

The variability in the rate of biogas production among the 100 anaerobic digesters may have been due to differences in their operating conditions (Kim *et al*., 2021).

The rate of biogas production is determined by the relationship between biogas production and the amount of substrate used for the production process (Yang *et al*., 2022).

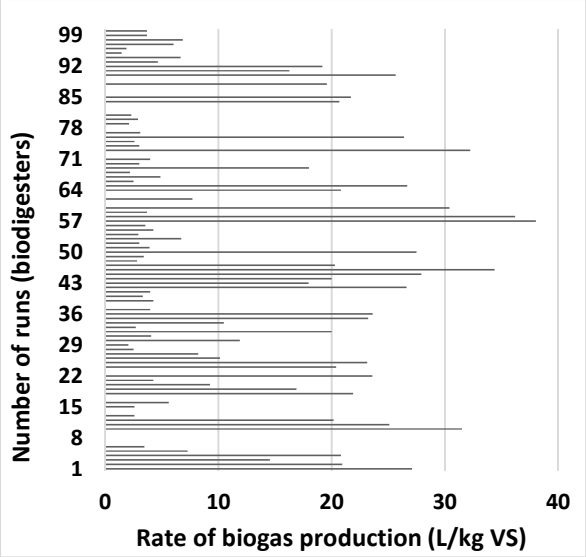

Fig 2: Rate (L/Kg VS) of biogas produced in the 100 bio-digesters

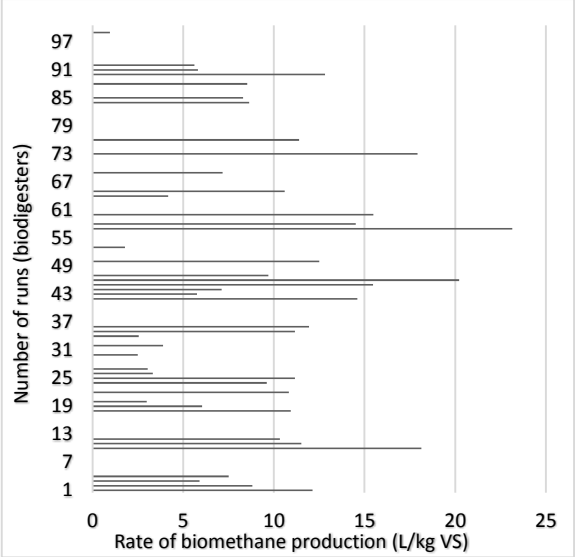

Fig 3: Rate (L/Kg VS) of methane produced in the 100 bio-digesters

Various studies have demonstrated that the rate of biogas production following anaerobic digestion of food waste and rumen content can vary as a result of differences in feedstock composition, operating conditions and other variables (Khalid *et al*., 2021; Fernandez-Lopez *et al*., 2022).

Rate of bio-methane production: The rate of methane (CH_4) production between bio-digester 1 and bio-digester 100 ranged from 0 – 23.14 L/Kg VS (Figure 3). The highest rate of methane production (23.14 L/Kg VS) was recorded in bio-digester 57 followed by bio-digester 46 (30.21 L/Kg VS), bio-digester 10 (18.13 L/Kg VS), bio-digester 73 (17.93 L/Kg VS), bio-digester 60 (15.49 L/Kg VS), bio-digester 45 (15.47 L/Kg VS), bio-digester 58 (14.52 L/Kg VS), bio-digester 90 (12.83 L/Kg VS) and bio-digester 1 (12.14 L/Kg VS). Bio-digester 57, which generated the highest rate of bio-methane production was operated at a food waste of 0.30 kg, rumen content of 0.30 kg, water content of 0.40 kg, temperature of 34.0oC, pH of 9.0, bio-digester agitation of 4 times/day and a retention time of 32 days. Variations in the rate of methane production among the 100 anaerobic digesters may have been due to differences in their operating conditions (Smith *et al*., 2020). Methane production rate is a parameter used to describe the relationship between biomethane production and the substrate used. It is the volume of methane in the cumulative biogas generated per weight of volatile solid content of the substrates (Yang *et al*., 2022). In previous studies, the rate of methane production following anaerobic digestion of food waste and rumen content have been shown to vary as a result of the composition of the feedstock, operating conditions and other variables (Slopiecka *et al*., 2022).

Rate of substrate degradation: The rate of substrate degradation between bio-digester 1 and bio-digester 100 ranged from 0 – 79.20% (Figure 4). The highest rate of substrate degradation (79.20%) was recorded in bio-digester 57 followed by bio-digester 10 (78.50%), bio-digester 73 (76.40%), bio-digester 60 (68.60%), bio-digester 46 (67.40%), bio-digester 58 (67.00%), bio-digester 11 (65.80%), bio-digester 90 (63.40%) and bio-digester 45 (62.20%). Bio-digester 57, which demonstrated the highest rate of substrate degradation was operated at a food waste of 0.30 kg, rumen content of 0.30 kg, water content of 0.40 kg, temperature of 34.0oC, pH of 9.0, bio-digester agitation of 4 times/day and a retention time of 32 days.

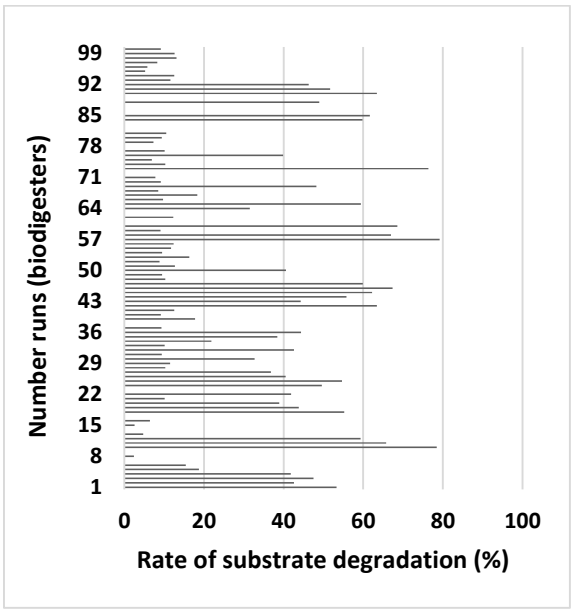

Fig 4: Rate of substrate degradation (%) inside the 100 bio-digesters

The variation in substrate degradation among 100 anaerobic digesters was due to differences in operating conditions (Mirmohammadi *et al*., 2022). Substrate degradation is the amount of substrate removed or converted to biogas during anaerobic digestion (Ghosh and Singh, 2015). Previous studies reported varying rates of substrate degradation for food waste and other substrates, depending on their composition and digestion conditions (Zhang *et al*., 2021).

Conclusion: This study highlights the significant impact of process parameters on the rate of biogas and bio-methane production, as well as substrate degradation, during anaerobic digestion of food waste and rumen content. It further demonstrates that anaerobic co-digestion of food waste and rumen content yields better results than anaerobic mono-digestion. This study contributes to the existing scientific knowledge on the optimization of anaerobic digestion processes, providing valuable insights for future research and practical applications in the renewable energy and waste management sectors.

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