



Physicochemical Characteristics and Potentials of Kitchen Food Waste, Untreated Food Effluent, and Cow Rumen Content as Substrates for Biogas Production via Anaerobic Digestion

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ABSTRACT: Anaerobic digestion (AD) stands as a promising solution for organic waste management and renewable energy production. However, its effectiveness relies on substrate selection and an understanding of biogas production dynamics. Hence, the objective of this paper was to evaluate the physicochemical characteristics and potential of kitchen food waste (KFW), untreated food effluent (UFE), and cow rumen content (RC) as substrates for biogas production via anaerobic digestion (AD) using appropriate standard methods. Substrates were physicochemically characterized and monitored over 35 days for biogas yield, pH variation, and nutrient dynamics. The co-digestion bioreactor yielded the highest cumulative biogas volume (13000.97 ml/kg VS), followed by RC (11708.97 ml/kg VS), KFW (8360.29 ml/kg VS), and UFE (4522.26 ml/kg VS). The highest total organic carbon (TOC) removal (97.47%) was recorded in the RC bioreactor. Microbial analysis revealed viable anaerobic bacteria ranging from $1.26\text{--}2.44 \times 10^4$ CFU/g in KFW, $1.5\text{--}6.4 \times 10^3$ CFU/mL in UFE, and $2.93\text{--}5.71 \times 10^4$ CFU/mL in RC. Biogas production was significantly influenced by substrate composition and digestion time ($p < 0.05$). Changes in nitrate, phosphate, and TOC concentrations confirmed active microbial metabolism and nutrient utilization. These findings highlight the efficiency of co-digestion in enhancing biogas yield and promoting sustainable organic waste management.

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Energy serves as a fundamental component driving the development activities of human civilization. However, the predominant reliance on non-renewable energy sources, such as fossil fuels (coal, gas, uranium), poses a significant challenge due to their finite nature and the escalating demands driven by population growth and urbanization (Dhanalakshmi *et al.*, 2012). According to estimates by the World Coal Association and British Petroleum, coal and natural gas reserves are anticipated to be depleted within 150 and 52 years, respectively. Moreover, the combustion

of fossil fuels has contributed to the accumulation of greenhouse gases, exacerbating global warming. In response to the looming energy crisis and environmental concerns, there has been a growing focus on renewable energy sources as sustainable alternatives. Renewable energy sources, offer solutions to mitigate environmental degradation and address energy security challenges. Among these sources, rumen digesta, a biomass derived from the microbial digestion occurring in the rumen of animals, holds the potential for renewable energy

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production (Al Mamum *et al.*, 2018). Rumen digesta, often considered waste, is typically discarded, leading to environmental pollution. However, its recycling presents an opportunity for sustainable waste management and energy production. Anaerobic digestion (AD) emerges as a key method for recycling rumen digesta to produce biogas, leveraging the presence of cellulolytic and methanogenic bacteria inherent in rumen microbial communities (Al Mamum *et al.*, 2018). This process mirrors microbial fermentation occurring naturally in the rumen, facilitating the conversion of organic matter into biogas.

Biogas, a combustible gas primarily composed of methane (CH_4) and carbon dioxide (CO_2), is generated through the anaerobic fermentation of organic matter (Al Mamum *et al.*, 2018). Biogas, and its refined form biomethane, hold promise as renewable energy carriers, capable of substituting fossil fuels and mitigating greenhouse gas emissions across various sectors, including energy, transportation, agriculture, and waste management (Wechselberger *et al.*, 2023). The utilization of biogas represents a sustainable approach to meet the escalating energy demands while simultaneously addressing waste management and environmental concerns. With the potential to convert various organic wastes into energy, biogas production is poised to play a significant role in the global energy landscape, with projections estimating a substantial increase in production by 2030. Hence, the objective of this paper is to evaluate the potentials of kitchen food waste (KFW), untreated food effluent (UFE), and cow rumen content (RC) as substrates for biogas production via anaerobic digestion (AD).

MATERIALS AND METHODS

Collection and pretreatment of the waste substrates: Kitchen food wastes (cooked rice, yam, beans, processed cassava, bread, fish bone, banana peels, pineapple peels, and orange peels), collected from food vendors within the University of Port Harcourt were homogenized to form a single substrate. Untreated effluent and 1 kg of rumen content was collected from a food manufacturing company and an abattoir respectively, in Choba, Port Harcourt. The rumen content was mixed with 3 litres of tap water, supplemented with D-glucose to encourage microbial growth and was loaded into a 4-litre capacity anaerobic digester. The rumen fluid was obtained from it after 1 month.

Physicochemical characterization of the waste substrates: Moisture content, total solid, volatile

solid, and ash content, were determined using the Gravimetric method outlined by Liotta *et al.* (2014).

The total organic carbon (TOC) content of the substrates was assessed by High-Temperature Combustion method as described by Erdogan *et al.* (2015).

The total nitrogen content in the substrates was determined using the Kjeldahl method, following the protocol described by Qiu *et al.* (2023).

Total nitrate content in the substrates was assessed using the Griess-Saltzman method, as detailed by Zhang *et al.* (2020).

Total phosphorus and total phosphate content in the substrates were determined using the Persulfate Digestion method, outlined by Takashima (2019). Total potassium content in the substrates was measured using Flame Photometry, following the protocol described by El-Nahhal *et al.* (2014).

The Chemical Oxygen Demand (COD) of the substrates was determined using the Dichromate method, as described by Ma (2017).

Determination of Heavy Metals in the waste substrate: Heavy metal content including copper, zinc, nickel, lead, and cadmium in the substrates was determined using the Atomic Absorption Spectroscopy (AAS) method outlined by Islam *et al.* (2016).

Determination of physicochemical changes in the biodigester: The changes in Total organic carbon (TOC), Total nitrate (NO_3^-) and Total phosphate (PO_4^{3-}) of the biodigester slurry were determined at 5-day intervals as explained in the previous sections.

Determination of PH: The pH of the biodigester content was determined using a digital pH meter immersed in the biodigester slurry samples.

Configuration and operation of the anaerobic digester: A plastic digester with a cylindrical shape was used (Plate 1). The digester had a volume capacity of 4 litres, out of which 3.5 litres were used for loading the substrate, leaving a headspace volume of 0.5 litres. The opening of the plastic container used as the biodigester served as the inlet for substrate addition. An outlet for biogas collection and slurry sample-collection port were attached to the lid of the biodigester opening. A 30ml syringe was attached to the sample collection port for the collection of slurry samples from the biodigester while maintaining the anaerobic nature of the biodigester. A CO_2 -removing

unit containing alkaline (KOH) water was connected to the biogas outlet of the anaerobic digester setup according to Dhanya *et al.* (2020). A temperature of 37°C maintained throughout. Heating for the digesters was provided by four 60-watt incandescent bulbs installed inside a plastic box (100cm x 60 cm) that housed the digesters. A digital thermostat was also installed inside the plastic box housing the digesters to ensure a stable temperature within the desired range and maintain operational consistency. The inner bottom lining of the plastic box was padded with a black carbon foam covered with a brown carton to enhance heat retention, temperature stability within the digester system. The feed for anaerobic digestion (Table 1) was loaded into the respective

digesters. Biogas production was monitored for 35 days at regular (5 days) intervals using the alkaline water displacement method. Biogas generated in the biodigester was evacuated from the digester and allowed to pass through the CO₂-removal unit, where CO₂ was absorbed by the alkaline water. The volume of alkaline water displaced was measured and considered to be equivalent to the volume of biogas generated in the biodigester.

Experimental design for anaerobic digestion of selected organic substrates: The setup involved four distinct anaerobic digestion (AD) systems, each designated with a specific substrate (Table 1).

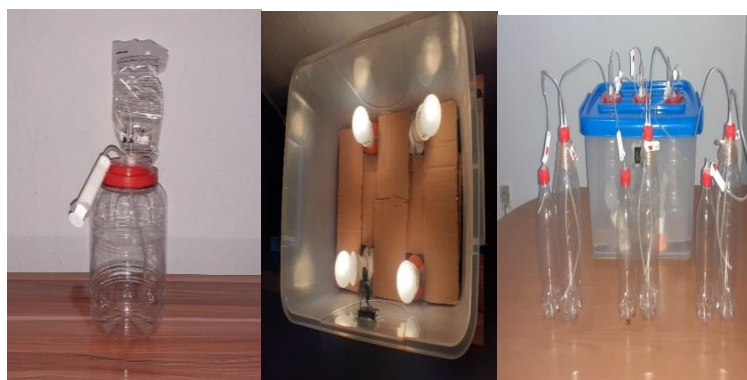


Plate 1: Biodigester with biogas outlet & slurry sample collection syringe (left); Heat-retention plastic box (middle); CO₂-remover unit (the bigger bottle) connected to a displaced alkaline water collector (the smaller bottle) (right)

Table 1: Experimental design for anaerobic digestion of selected organic substrates

AD System	Substrate	Substrate quantity Solid (kg)	Rumen Rumen (kg)	Water Content (kg)	Total (kg)
AD _{KFW}	KFW	0.69	1.5	1.15	3.0
AD _{UFE}	UFE	0.69	1.5	1.15	3.0
AD _{RCC}	CRC	0.69	1.5	1.15	3.0
AD _{COS}	KFW + UFE + RC	0.69	1.5	1.15	3.0

KFW=Kitchen Food Waste; UFE=Untreated Food Effluent; RC=Rumen Content; KFW + UFE + RC (1:1:1) = Co-substrates

Determination of biogas production: The cumulative biogas volume was measured at 5-day intervals, using alkaline water displacement method. Combustion qualitative test was used to confirm the flammability of the biogas produced

The rate of change of TOC removal with time (TOC_{RT}) and the rate of cumulative TOC removal with time (TOC_{CT}) were determined to assess the biodegradability of the substrate inside the biodigester (equations 1 and 2 respectively) (Alhothali *et al.*, 2022).

$$\%TOC_{RT} = \frac{[TOC_{PT} - TOC_{CT}]}{TOC_{PT}} * 100 \quad (1)$$

Where; TOC_{PT} = TOC at the previous time; TOC_{CT} = TOC at the current time

$$\%TOC_{RT} = \frac{[TOC_{CPT} - TOC_{CCT}]}{TOC_{CPT}} * 100 \quad (2)$$

Where; TOC_{CPT} = cumulative TOC at the previous time ; TOC_{CCT} = cumulative TOC at the current time

Statistical Analysis: Analysis of variance (ANOVA) was employed to determine the impact of substrate on process performance concerning biogas yield.

RESULTS AND DISCUSSION

Physicochemical properties of the waste substrates: Untreated Food Effluent exhibited the highest moisture content, indicating a significant water

content in the substrate, followed by Kitchen Food Waste and Rumen Content (Table 2). Moisture content can facilitate the breakdown of organic matter during anaerobic digestion, promoting biogas production (Rasapoor *et al.*, 2020). However, excessive moisture levels can also lead to poor substrate homogenization and reduced gas yield per unit of substrate (Rocamora *et al.*, 2020). Untreated Effluent, with the highest moisture content, may require additional dewatering or adjustments in feedstock composition to optimize biogas production (Kasinath *et al.*, 2021). Rumen Content demonstrated the highest total solid content. Total solids provide the raw materials necessary for microbial activity and methane generation during anaerobic digestion (Syaichurrozi and Sumardiono, 2014). However, excessively high total solid content can hinder process performance, require dilution or mix with other substrates to achieve optimal conditions (Wu *et al.*, 2021). Kitchen Food Waste showed the highest volatile solid content. Volatile solids represent the portion of organic matter available for microbial degradation and subsequent biogas production (Issah and Kabera, 2021). Kitchen Food Waste, with the highest volatile solid content, offers a substantial source of readily biodegradable material for methane generation. Efficient utilization of volatile solids can enhance biogas yields and process stability, provided adequate process control measures are in place

(Noraini *et al.*, 2017). High ash content, as observed in Rumen Content, indicated the presence of inert materials that contribute little to biogas production (Abbas *et al.*, 2023). While some ash content is inevitable in organic substrates, excessively high levels may require pretreatment or blending with other materials to optimize biogas production efficiency (Mishra *et al.*, 2021). Kitchen Food Waste had the highest values for parameters like Total Organic Carbon (TOC), Total Nitrate (NO_3^-), and Chemical Oxygen Demand (COD), compared to the other substrates presents a favourable substrate for biogas production due to its rich organic composition and nutrient content. (Qi *et al.*, 2020; Marin *et al.*, 2022). The presence of heavy metals in waste substrates can impact biogas production and the quality of digestate (Borek *et al.*, 2015). While some metals may act as catalysts for anaerobic digestion processes, high concentrations can inhibit microbial activity and pose environmental risks (Chen *et al.*, 2014). Rumen Content may contain higher levels of heavy metals due to dietary intake by the cow, while Kitchen Food Waste might accumulate metals from food packaging or cooking utensils. Proper monitoring and management of heavy metal levels are essential to ensure the sustainability and safety of biogas production and utilization of digestate in agricultural or other applications.

Table 2: Physicochemical properties of the waste substrates

Physicochemical Properties	KFW	UFE	RC
Moisture Content [%]	76.3	89.4	74.2
Total Solid [%]	23.7	10.6	25.8
Volatile Solid [%]	94.7	97.5	74.9
Ash Content [%]	5.3	2.5	25.1
Total Organic Carbon (TOC) [mg/kg]	45000.8	15000.3	39000.4
Total Nitrogen (TN) [mg/kg]	2050.2	1146.5	2156.9
Total Nitrate NO_3^- [mg/kg]	352.1	258.3	173.8
Total Phosphorus (TP) [mg/kg]	721.4	536.6	648.2
Total Phosphate (PO_4^{3-}) [mg/kg]	216.3	150.9	263.8
Total Potassium (TK) [mg/kg]	3192.2	1873.9	4016.5
Chemical Oxygen Demand (COD) [mg/kg]	15204.3	10931.5	12409.7
Copper (Cu) [mg/kg]	10.7	5.9	8.3
Zinc (Zn) [mg/kg]	52.3	35.8	46.6
Nickel (Ni) [mg/kg]	2.6	1.4	3.8
Lead (Pb) [mg/kg]	1.3	2.7	1.9
Cadmium (Cd) [mg/kg]	0.0	0.5	0.8

Cumulative biogas generation and yield from the substrates: The volume of cumulative biogas generation (Fig. 1) and the variations in biogas yield (Fig. 2) from the various substrates over 35 days were shown. Initially, on Day 1, no detectable biogas yield was observed, indicating an initial lag phase in anaerobic digestion where microbial activity is established (López-Aguilar, *et al.*, 2023). From Day 15 onwards, the appearance of biogas yield signifies the onset of biogas production, with methanogenic

microorganisms converting volatile fatty acids into methane and carbon dioxide (Zabranska and Pokorna, 2018). Substrates such as Rumen Content and Co-Substrates consistently exhibited higher biogas yield compared to the Kitchen Food Waste and Untreated Effluent, suggesting favorable conditions for methanogenesis.

Data analysis: The analysis of variance (ANOVA) (Table 3) revealed significant variations in biogas

yield among both substrates and time points. This suggests that the choice of substrate and the progression of time significantly influence biogas yield through anaerobic digestion (Li *et al.*, 2019). The observed differences underscore the importance of substrate composition and process optimization in maximizing biogas yield in anaerobic digestion systems (Hobbs *et al.*, 2018; Ahmad *et al.*, 2024).

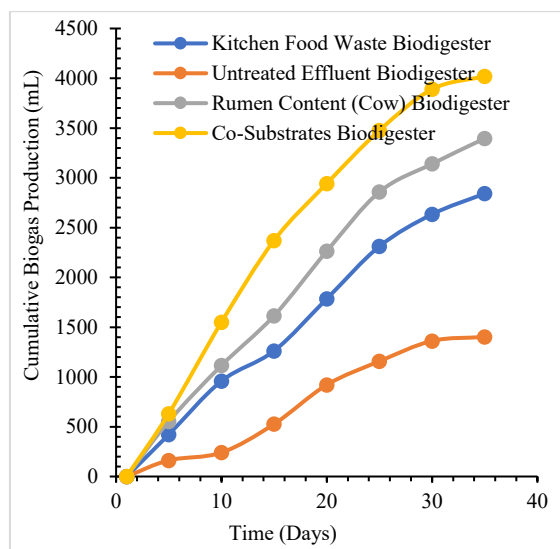


Fig. 1: Volume of cumulative biogas generated by the substrates

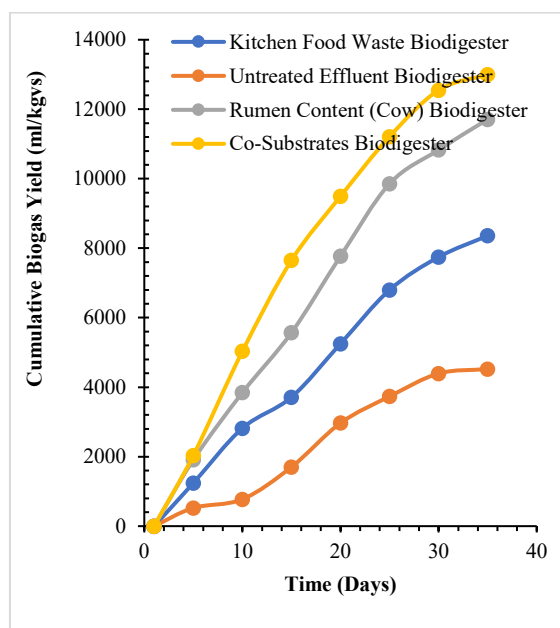


Fig. 2: Cumulative biogas yield of the substrates

pH dynamics during biogas production: Throughout the 35-days, variations in pH levels were observed among the different biodigesters (Figure 3). While all biodigesters initially started with slightly acidic pH values ranging from 6.5 to 6.9, they exhibited distinct

pH dynamics over time. The Kitchen Food Waste Biodigester consistently showed the lowest pH values compared to the other biodigesters, while the Co-Substrates Biodigester generally maintained higher pH levels. The observed fluctuations in pH levels during biogas production can be attributed to the complex biochemical processes occurring within the biodigesters (Hagos *et al.*, 2017). Initially, the slight decrease in pH may result from the acidogenic phase, where organic matter is broken down into volatile fatty acids, by acidogenic bacteria, leading to the release of acidic byproducts (Ramos-Suarez *et al.*, 2021).

Additionally, the production of carbon dioxide during fermentation processes can lead to the formation of carbonic acid, further lowering the pH of the digestate (Fernández *et al.*, 2015). Towards the later stages of the experiment, the observed variations in pH levels may be attributed to shifts in microbial activity and substrate availability. As the availability of fermentable organic matter decreases and methanogenic bacteria become dominant, the pH levels may start to stabilize or even increase slightly due to the consumption of volatile fatty acids and the production of alkaline compounds such as ammonia during the methanogenic phase (Dahiya *et al.*, 2015; Wang *et al.*, 2014).

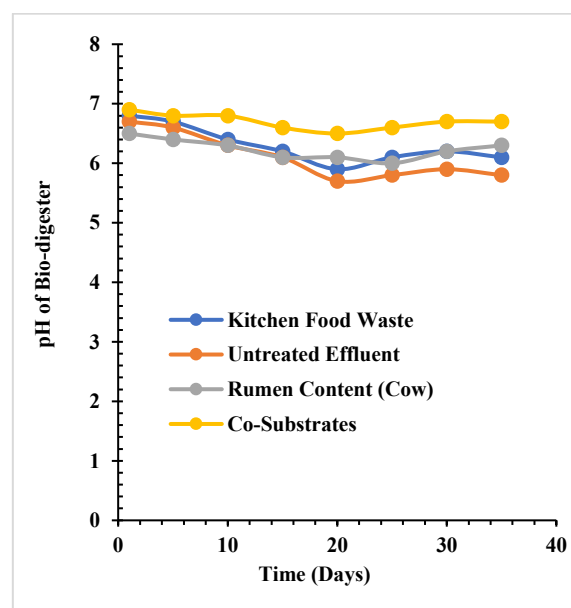


Fig.3: pH dynamics during biogas production

Dynamics of total organic carbon (TOC) during biogas production: The dynamics of Total Organic Carbon (TOC) levels during biogas production varied among the different substrates over the 35 days (Figure 4). Each biodigester initially started with distinct TOC values, and these values gradually

decreased over time. Additionally, the rate of change of TOC removal with time (TOC_{RT}) as shown in Figure 5 and the rate of cumulative TOC removal

with time (TOC_{CT}) as shown in Figure 6 differed among the substrates, reflecting variations in organic matter degradation rates (Nwokolo *et al.*, 2020).

Table 3: ANOVA of Biogas Yield of the Different Substrates

Source of Variation	SS	df	MS	F	P-value	F crit
Rows	342872860.9	7	48981837.28	24.95170067	8.0865E-09	2.487577704
Columns	129168150.1	3	43056050.04	21.93306197	1.12911E-06	3.072466986
Error	41224387.73	21	1963066.082			
Total	513265398.8	31				

The observed reductions in TOC levels across all biodigesters indicate the degradation of organic matter during the biogas production process (Estrada-Arriaga *et al.*, 2021; Li *et al.*, 2017). Initially, the high TOC values represent the concentration of organic carbon in the substrates, which serve as the feedstock for anaerobic digestion. As microbial activity progresses, organic compounds are broken down into simpler molecules, leading to a decrease in TOC levels (Chen *et al.*, 2022). The differences in TOC dynamics among the substrates may be attributed to variations in substrate composition, microbial populations, and fermentation pathways (Rabii *et al.*, 2019). Biodigesters containing substrates with higher initial TOC values may exhibit more rapid organic matter degradation, resulting in faster TOC removal rates (Lee *et al.*, 2020). Conversely, biodigesters with lower initial TOC values may undergo slower degradation processes, leading to comparatively slower TOC removal rates (Cucina *et al.*, 2021). The TOC_{RT} values indicate the percentage of TOC removed from the digesters at each time point, providing insights into the efficiency of organic matter degradation over time (Qi *et al.*, 2022; Xiao *et al.*, 2020). Higher TOC_{RT} values suggest more rapid TOC removal, while lower values indicate slower degradation rates. Similarly, the TOC_{CT} values represent the cumulative percentage of TOC removed from the digesters throughout the experiment, reflecting the overall efficiency of biogas production and organic matter conversion (Li *et al.*, 2017).

Dynamics of nitrates (NO_3^-) concentration during biogas production: The dynamics of Nitrate (NO_3^-) concentrations during biogas production varied among the different substrates over the 35 days (Figure 7). Each biodigester initially started with distinct Nitrate concentrations, and these concentrations gradually decreased over time indicating the utilization of nitrates by microbial communities during the anaerobic digestion process (Cai *et al.*, 2021). Initially, the high Nitrate concentrations in the substrates serve as potential

electron acceptors for microbial respiration (Pang & Wang, 2021).

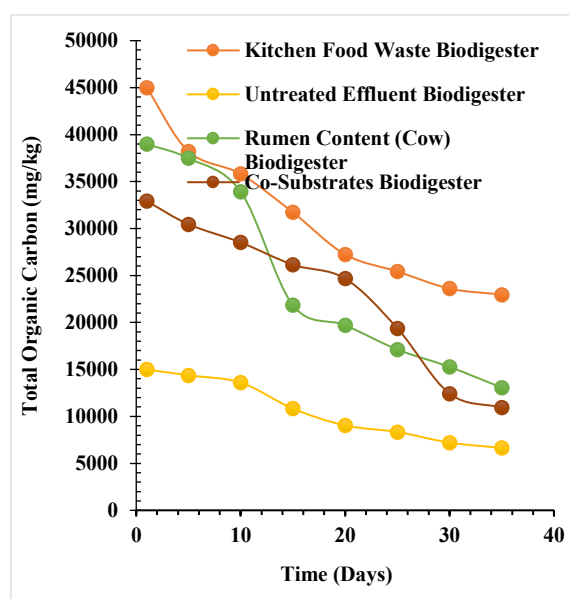


Fig. 4: Dynamics of TOC during biogas production

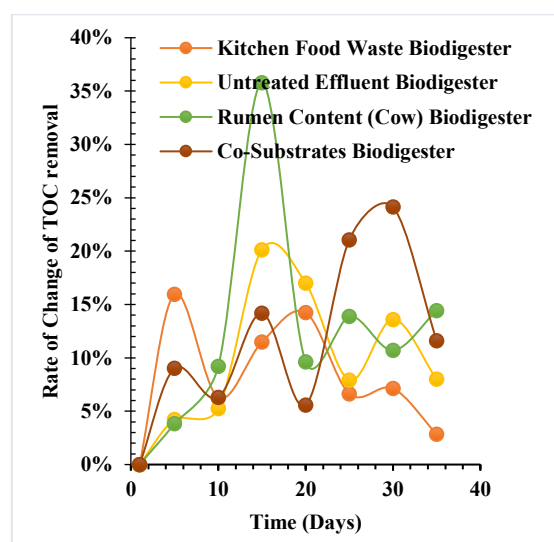


Fig. 5: Rate of Change of TOC removal with time inside the anaerobic digesters

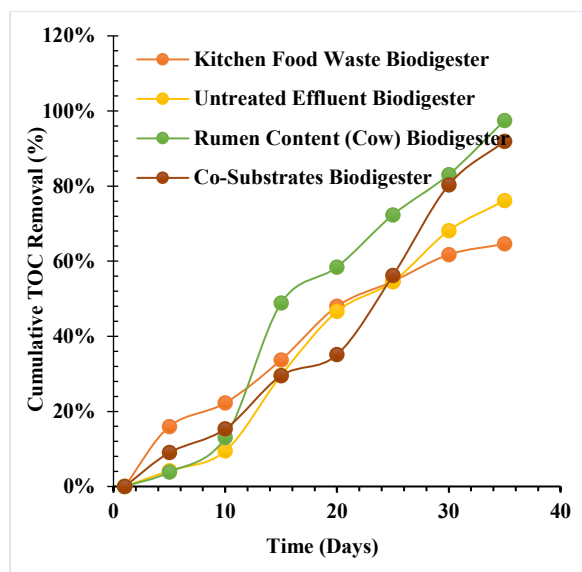


Fig. 6: Rate of Cumulative TOC removal with time inside the anaerobic digesters

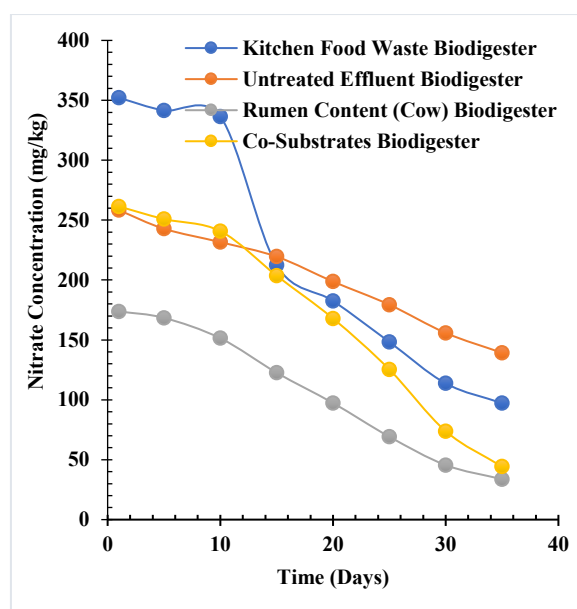


Fig. 7: Dynamics of nitrates (NO_3^-) concentration during biogas production

As microbial activity progresses, nitrates are reduced to nitrites, and further to nitrogen gas (N_2), resulting in a decrease in Nitrate concentrations (Jonassen *et al.*, 2022). Biodigesters containing substrates with higher initial Nitrate concentrations may experience more significant reductions in Nitrate levels over time, reflecting higher rates of microbial nitrate utilization. Additionally, differences in microbial community composition and metabolic pathways may influence the efficiency of Nitrate reduction among the substrates (Sun *et al.*, 2020; Xu *et al.*, 2018).

Dynamics of phosphates (PO_4^{3-}) concentration during biogas production: The dynamics of Phosphate (PO_4^{3-}) concentrations during biogas production varied among the different substrates over the 35 days (Figure 8). Each biodigester initially started with distinct Phosphate concentrations, and these concentrations gradually decreased over time. The observed reductions in Phosphate concentrations across all biodigesters indicate the utilization of phosphates by microbial communities during the anaerobic digestion process (Belostotskiy *et al.*, 2015; Keating *et al.*, 2016). Initially, the high Phosphate concentrations in the substrates serve as essential nutrients for microbial growth and metabolism (Mao *et al.*, 2019; Xu *et al.*, 2018). As microbial activity progresses, phosphates are assimilated into microbial biomass or precipitated as insoluble compounds, leading to a decrease in Phosphate concentrations in the liquid phase (Flores-Alsina *et al.*, 2016; Ma *et al.*, 2018). Biodigesters containing substrates with higher initial Phosphate concentrations may support more active microbial communities capable of efficient phosphate utilization, leading to faster decreases in Phosphate levels over time. Conversely, substrates with lower initial Phosphate concentrations may exhibit slower rates of Phosphate reduction, resulting in comparatively slower decreases in Phosphate concentrations.

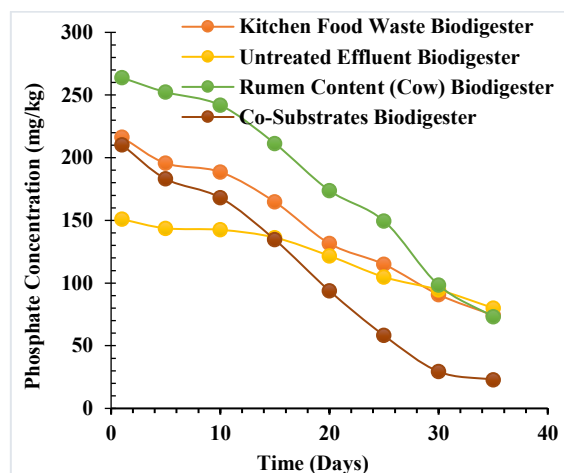


Fig. 8: Dynamics of PO_4^{3-} concentration during biogas production

Conclusion: This study emphasizes the potential of co-digestion strategies and rumen-derived inocula to enhance biogas production while promoting sustainable waste management. Therefore, anaerobic digestion technology for organic waste management, contributing significantly to waste diversion from landfills and the reduction of greenhouse gas emissions should be adopted.

Declaration of Conflict of Interest: The authors declare that no known conflict of interest or personal relationships that could have influenced the work reported in this paper.

Declaration of Data Availability: Data are available on request from the corresponding author.

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