

SYSTEMATIC REPLACEMENT OF FRESHWATER WITH AQUACULTURE EFFLUENTS FOR ENHANCED ANAEROBIC DIGESTION OF FOOD WASTE

*Erewari Ukoha-Onuoha, Ngozi Faith Udenze

Department of Agricultural and Environmental Engineering, Faculty of Engineering, Rivers
State University PMB 5080, Port Harcourt, Nigeria.

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*Corresponding Author

Erewari Ukoha-Onuoha

Department of Agricultural and
Environmental Engineering,
Faculty of Engineering, Rivers
State University PMB 5080, Port
Harcourt, Nigeria.

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ABSTRACT

This research examined the replacement of freshwater in dry anaerobic digestion (AD) of food waste (FW) with brood stock effluent (BSE). A laboratory experiment was conducted in unstirred single phase anaerobic digestion (U-SPAD) system with five different mix-ratios of BSE to tap water (TW): BSE100%, BSE75%:TW25%, BSE50%:TW50%, BSE25%:TW75%, and TW100%. A retention time of 30 days was maintained under mesophilic conditions. Biogas was measured daily, while other parameters including pH, temperature, chemical oxygen demand (COD), and C/N ratio were determined every five (5) days interval. Biogas composition and calorific values were determined at the end of the 30-days. All parameters were measured using standard methods. The biogas yield ranged from 0.38 – 0.52 m³/kg VS while COD reduction ranged from 61 – 68%. Biogas yield

correlated strongly with %COD reduction ($r = 0.88$). However, ANOVA analysis showed no significant difference in biogas yield among mix-ratios ($P = 0.86$, $F = 0.3234$ at $p < 0.05$). Methane percentage of 64.62 – 85.48% and calorific value of 13.96 – 27.44 MJ/kg were obtained for the various mix-ratios. BSE100% with the highest biogas yield did not produce the most biomethane. BSE75%:TW25% showed the highest methane and calorific value due to lower concentrations of non-combustible gases (nitrogen 4.36% and water vapour 3.64%)

compared to BSE100% (nitrogen 6.64 % and water vapour 5.1%). The results demonstrate that BSE can substitute freshwater in AD, reducing fresh water consumption while enhancing methane yield and energy recovery. This aligns with Sustainable Development Goals 6 (Clean Water and Sanitation), 7 (Affordable Energy), and 13 (Climate Action).

KEYWORDS: Calorific Value, Circular Economy, Environmental Challenge, Freshwater Scarcity, Biogas Yield.

1. INTRODUCTION

Anaerobic digestion (AD), aquaculture production, food waste generation, and freshwater availability are important global challenges that are closely related to food security, environmental sustainability, and renewable energy production. Worldwide, aquaculture has grown rapidly to meet the increasing demand for protein, reaching about 126 million tonnes live weight in 2022 (Mair et al., 2023). At the same time, food waste remains a pressing global issue, with approximately 1.3 billion tonnes of food lost or wasted annually, accounting for roughly one-third of all food produced (Rai et al., 2025). This food waste, when disposed of improperly, releases large amounts of greenhouse gases, estimated at about 3.3 billion tonnes of CO₂ equivalents annually (Rai et al., 2025). In addition, freshwater scarcity is becoming more serious. Freshwater makes up only about 2.5% of the world's total water, and only around 1% is easily available for use (UNESCO, 2020). The combination of rapid aquaculture growth, high levels of food waste, and limited freshwater resources shows the need for practical and sustainable solutions to address these connected challenges.

Anaerobic digestion (AD) is a process that breaks down organic materials biologically in the absence of oxygen and produces biogas (mostly methane) as well as a nutrient-rich residue called digestate. This is typically done for different purposes such as organic waste management, renewable energy generation, and the production of soil conditioners. Anaerobic digestion could either be wet or dry, batch or continuous and mesophilic or thermophilic. Wet AD operates with a total solid content of less than 15%, requiring water or liquid co-substrates for slurry formation (Oduor et al., 2022). Wet AD is commonly used for sewage sludge and food waste treatment. While dry digesters use high-solid feedstocks (15–40% total solids), reducing the need for water (Liu et al., 2025). However, dry or wet AD, fresh water is an indispensable resource for the AD system efficiency.

Fresh water in AD plants serves multiple essential functions. Firstly, it acts as a carrier for the organic matter that is being degraded. This action boosts efficient bacterial colonization of the substrate and the access of the methanogens (Kothari *et al*, 2014). Water also keeps the pH at the required level, vital substances can be transported, and the temperature profile in the digester can be easily observed (Appels *et al*, 2008). Additionally, water helps to dilute the concentration of toxic compounds such as ammonia, hydrogen sulfide, and heavy metals that could inhibit the growth of microbes in the system. Furthermore, in the co-digestion of high-solids or nutrient-rich waste such as animal manure and food waste, the addition of freshwater is a must to avoid system failure due to over-acidification or sludge build-up. The use of fresh water is also crucial in the prevention of the introduction of pathogens. However, freshwater is a scarce resource.

The rapid expansion of aquaculture has created substantial environmental challenges through the generation of significant nutrient-rich effluents. In developing countries like Nigeria, untreated aquaculture effluents are frequently discharged into surface water bodies (Sampantamit *et al.*, 2020). The constituents of aquaculture effluent especially suspended solids, organic matter, and nutrients such as nitrogen and phosphorus results in eutrophication, oxygen depletion, and siltation of receiving water bodies (Browdy *et al*, 2001; Naylor *et al*, 2000; Camargo and Alonso, 2006). These impacts lead to significant changes in aquatic ecosystems, altering species composition, reducing biodiversity, and deteriorating water quality, making it unsuitable for drinking, recreation, or other human uses (Camargo and Alonso, 2006; Inkani *et al*, 2025). The effluents can also contribute to the spread of invasive species and pathogens, further disrupting aquatic ecosystems as well as pose serious health risks and food safety problems for downstream users (Peeler *et al*, 2011; Tuan *et al*, 2013; Henriksson *et al*, 2018; Al-Salmi, 2023).

Another major environmental challenge is food waste. Each year, about 1.3 billion tons of food is wasted and over 95% of this waste ends up in landfills where it creates methane and other greenhouse gases exacerbating climate change (Melikoglu *et al*, 2013). It's worth mentioning that nutrient-rich aquaculture effluent and food waste with high organic content can work together as co-substrates in anaerobic systems (Paes *et al.*, 2025). The combined use of food waste and aquaculture effluent could boost biogas production while also mitigating the challenge on freshwater and environmental pollution.

Recent studies have examined the use of aquaculture effluents as feedstock in anaerobic digestion (AD) systems. Research shows that nutrient-rich aquaculture effluent and food waste with high organic content can serve as suitable co-substrates in anaerobic digestion (Paes et al., 2025). For example, Paes et al. (2025) studied the anaerobic digestion of aquaponic effluent mixed with cattle manure and reported improved biogas production. They also found that at certain mixing ratios, there was no need to add extra water. In a related study, Netshivhumbe et al. (2024) investigated fish sludge from recirculating aquaculture systems and observed that co-digestion with food waste and fruit/vegetable waste greatly increased biomethane yield, producing methane yields up to eight times higher than digesting fish sludge alone. Furthermore, Venugopal (2021) reported that combining aquaculture waste with other organic waste streams not only improves biogas production but also supports better waste management within a circular economy system. However, there is still limited research on how well aquaculture effluent can replace water in AD systems, the long-term stability of such co-digestion processes under different operating conditions, and how these systems can be scaled up for practical use in aquaculture-producing areas. These gaps highlight the need for more research, especially considering global concerns about freshwater scarcity, food waste management, and sustainable use of aquaculture byproducts.

This study focuses on the use of food waste and aquaculture effluent in anaerobic digestion to address related environmental and sustainability issues. The objectives of the study are: (1) to evaluate biogas production and system stability when aquaculture effluent is used as a partial or full replacement for water in co-digestion with food waste; (2) to examine the interaction between aquaculture effluent and food waste and determine the best mixing ratio for higher biomethane yield. The expected benefits of this research include reducing the use of potable water in AD systems, providing a useful way to manage aquaculture effluents, improving resource recovery from food waste through increased biogas and nutrient production, and promoting circular economy practices in aquaculture and waste management systems. This study also supports key Sustainable Development Goals, including clean water and sanitation (SDG 6), affordable and clean energy (SDG 7), responsible consumption and production (SDG 12), and climate action (SDG 13).

2. MATERIALS AND METHODS

2.1 Digester Feed Sample Collection and Experimental Set up

Food waste, aquaculture effluent and tap water were used as feed stock. The aquaculture effluent used was a brood stock effluent (BSE) collected from a fish farm in Port Harcourt, Rivers State, Nigeria (Latitude 4.9029° N, Longitude 7.0013° E). BSE samples were collected at different times during effluent discharge and mixed to form a composite sample. Food wastes were collected in bin bags from restaurants, markets, homes, catering companies, and hotels to obtain a representative municipal food waste sample. The wastes were delivered to the laboratory for sorting, segregation, pulverization and homogenization to ensure uniform composition before use in the digesters. Tap water was collected from the laboratory.

A laboratory-scale dry unstirred single phase AD (U-SPAD) system was set up using large 18-litre plastic bottles as digesters. The digesters were loaded with fixed solid food waste (FW) of 30% and 70% water of a varying mix-ratio of broodstock effluent (BSE) and tap water (TW) as shown in Table 1. The food waste to water ratio was maintained at 3:7 consistent with report on optimum water level for dry anaerobic system (Kumar et al., 2024). The retention time was kept the same at 30 days for all mixed ratios. This also follows the report of Hartmann and Ahring (2006) and Mao et al., (2015). Each digester was manually mixed for homogenization just at the initial loading and thereafter the digesters were sealed and left to operate without stirring.

Table 1: Experimental Set-up.

Digester ID	Composition of mix ratio
A	FW+BSE 100%
B	FW+ BSE 75% + TW25%
C	FW+BSE 50%+ TW50%
D	FW + BSE 25% +TW75%
E	FW + TW100%

FW: Food Waste, BSE: Brood Stock Effluent and TW: Tap water.

2.2 Laboratory Analyses

A total of 30 digesters were set up for the 5 mix-ratios with each mix-ratio having six digesters with one digester being dismantled every 5 days interval. The initial and final pH, temperature, moisture content (MC), chemical oxygen demand (COD), Carbon (C) and nitrogen (N) of substrates were measured to monitor the physiochemical changes throughout

the experimental period at intervals of 5 days. Daily biogas production was also measured and the percentage composition of methane in the biogas was analyzed after 30 days. Temperature and pH were measured using a Multi meter (pH-W3988) while the calorific value of the biogas was determined using digital bomb calorimeter. Analytical methods of other parameters are presented in Table 2.

Table 2: Analytical Methods of Substrate Parameters.

Parameter of Substrate	Analytical method
COD	Open Reflux Method (APHA – 5520 B)
% Carbon content	Combustion Train Gravimetric
% Nitrogen content	Macro-kjeldahl method
Moisture content	Oven dry

2.3 Statistical Analysis

A single factor Analysis of variance (ANOVA) was used to determine the significance difference in the mean values of biogas production for the various mixed ratios of BWS and TW using MS Excel software. Differences were considered significant if $F > F_{critical}$ at 5% significance levels.

3 RESULTS AND DISCUSSION

3.1 Physicochemical Properties of AD feed stock and biogas production

Table 3 summarizes the physicochemical compositions of each individual feedstock, which include food waste (FW), brood stock effluent (BSE), and tap water (TW). Results showed that the pH of the tap water and the brood stock effluent were slightly acidic and similar to each other with pH values of 6.3 and 6.2 respectively. In relation to food waste, its pH value of 4.55 categorizes it as a much stronger acidic condition. All feed stocks had similar temperature values. However, the COD of food waste was much greater than BSE and TW. This is expected because food waste has a lot of organic matter, BSE contains less metabolic waste, and untreated tap water might contain organic matter. Moreover, the results of pH, temperature and COD corroborate the findings of Oduor *et al.* (2022) and the moisture content of food waste is within the range as listed by Selvam *et al.* (2021).

Table 3: Physicochemical Parameters of the individual feed stock before mixing.

Parameter	Tap Water	Brood Stock Effluent	Food Waste
pH	6.3	6.2	4.55
Temperature	28.8	28.9	28.3
Moisture Content (%)	100	100	84
COD (g/l)	0.008	92.48	151.68

The substrates' mean initial and final physicochemical properties for the different mix-ratios are presented in Table 4. Observably, the initial pH values of the substrates for all mixed ratios were acidic ($\text{pH } 4.52 \pm 0.35 - 4.58 \pm 0.45$), followed by a gradual increase to much milder acidic pH, ranging from $5.15 \pm 0.11 - 5.47 \pm 0.33$ by the 30-day retention time. The acidic pH is a common occurrence when food waste is the sole substrate and the pH is not adjusted (Kong et al, 2016, Shiyan et al., 2022, He et al., 2024, Moronkola et al., 2025, Muhammad et al., 2025). However, under established conditions for conventional single phase anaerobic digestion (SPAD), such acidic pH is not favourable for high biogas yield due to inhibition of methanogenesis (Kundu et al., 2017). Methanogens are commonly reported to perform optimally at pH band of 6.8 – 7.2 (Gerardi, 2003). However, Latif et al. (2017) reported that methane production inhibition at low pH conditions is due to step-wise dosing of acid that results in pH shock. And that with a gradual pH change over 24 hours, methane production occurred at low pH. Similarly, in this study, appreciable biogas yield ranging from 0.38 – 0.52 m^3/kg VS was obtained for the various mix ratios (Table 4). The persistent methane production observed implies that the bulk pH alone might not fully represent the actual functional conditions prevalent within the reactor and is attributable to several factors principal among these factors is the digester type used.

Table 4: Physicochemical Parameters of Cumulative Substrate Analysis After Mixing.

Digester ID	Initial pH	Final pH	Initial COD (g/l)	Final COD (g/l)	Initial C/N	Final C/N	Mean Biogas yield (m^3/kg VS)
A	4.55 ± 0.45	5.15 ± 0.11	490.02 ± 45	159.18 ± 87	26:2	21:2	0.52
B	4.53 ± 0.14	5.47 ± 0.33	462.98 ± 31	175.89 ± 63	14:4	10:5	0.44
C	4.52 ± 0.35	5.22 ± 0.13	426.21 ± 27	167.04 ± 89	19:4	16:3	0.36
D	4.54 ± 0.09	5.34 ± 0.14	426.35 ± 26	159.49 ± 75	18:2	18:1	0.38
E	4.58 ± 0.37	5.25 ± 0.11	459.46 ± 46	169.98 ± 74	28:1	23:1	0.42

In this work an unstirred single phase anaerobic digestion (U-SPAD) was used and one phenomenon that is prevalent in U-SPAD is vertical stratification. The absence of mechanical mixing facilitates natural phase separation and vertical stratification within the digester. As conceptually illustrated in Figure 1, U-SPAD maintained distinct functional layers, including scum, supernatant, active sludge, and digested sludge. These layers and the different

dominant biochemical activities in each layer replicates, to a degree, the functionalities of a two-phase anaerobic digestion (TPAD) system within a single reactor. TPAD creates favourable conditions for fermentation and methanogenesis in different digesters and generates higher energy yield (Feng et al., 2021, Simeonov et al., 2025). However, U-SPAD creates both fermentation and methanogenesis in a single digester but in different layers. The upper (scum and supernatant) layers of the U-SPAD were primarily associated with hydrolysis and acidogenesis because of the accumulation of readily degradable substrates and soluble organics. However, the absence of mixing slowed hydrolysis and acidogenesis and production of VFAs (Gerardi, 2003) mitigating reduction in the system bulk pH. An increase in the systems' bulk pHs were rather observed as shown in Table 4.

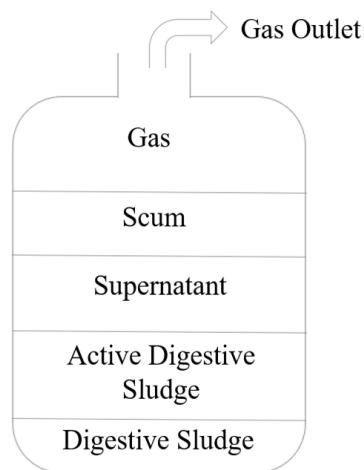


Figure 1: Schematic display of U-SPAD vertical stratification.

Within the active sludge layer, methanogenesis predominantly occurs because of the higher microbial density, improved biomass retention, and more favorable buffered micro-environmental conditions (Zhou et al., 2025). Micro-environments are common occurrence in anaerobic systems as microbes frequently organize themselves into biofilms and dense flocs, which establish localized physicochemical gradients. These gradients enable methanogens to endure within protected micro-niches, even when the surrounding bulk liquid remains acidic (Abera et al., 2024). Such micro-environments can sustain internal near-neutral pHs, thereby supporting methanogenic activity despite an unfavorable bulk environment (Zhou et al. 2025, Cayetano et al., 2022, Cong et al., 2021, Brileya et al., 2014). Methanogenic activity often involves VFA consumption. The observed increase in system bulk pH from strongly acidic to mildly acidic conditions (Table 4) lends credence to the notion of partial VFA consumption and biofilm internal buffering.

Smith et al. (2015) demonstrated the occurrence of methanogenesis in biofilms using low, medium, and high fouling membranes bioreactors and observed that COD removal in anaerobic digestion directly correlates with biofilm formation. Similarly the COD % reduction in this work correlated linearly ($r = 0.88$) with biogas production as shown in Figure 2. Considering that COD indicates the amount of organic matter in the substrate, the reduction supports the hypothesis regarding diminished biogas production due to a reduction in organic nutrients essential for microbial populations. Digester A, with the highest COD reduction, showed the highest biogas yield, while digester C, with the least COD reduction, recorded the lowest output. However, with $P = 0.86 (>> 0.05)$ and $F < F_{critical}$ as shown in Table 5 the mean biogas yields are not significantly different.

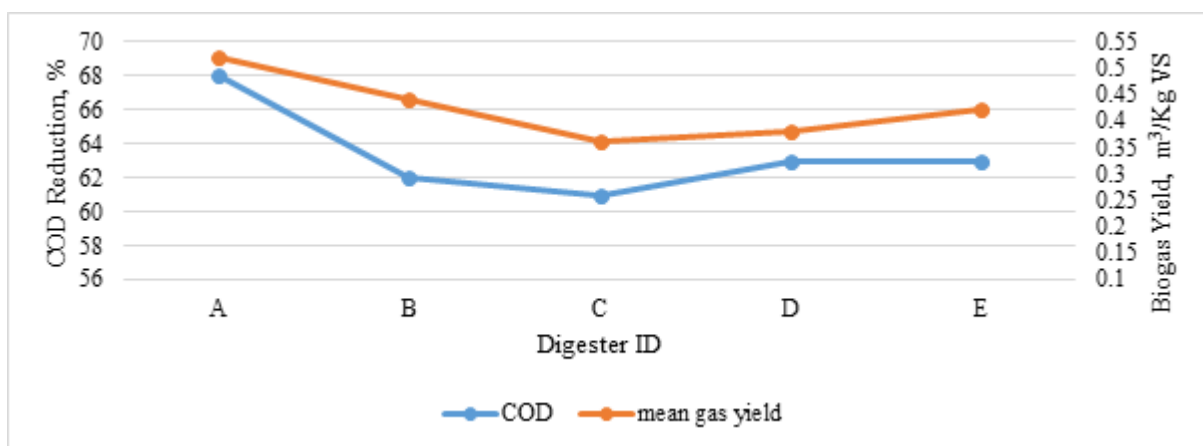


Figure 2: Relation Between COD Reduction and Mean Biogas Yield in U-SPAD System with Varying Mix-Ratio of BSE and TW.

Also, the observed increase in system bulk pH from strongly acidic to mildly acidic conditions (Table 4) further lends credence to the notion of partial VFA consumption and internal buffering. The specific composition of the food waste substrate, particularly its protein fraction, likely contributed to this behavior. The degradation of proteins releases amino groups ($-NH_2$) and ammonia (NH_3) which enhances alkalinity, thereby mitigating acid accumulation and facilitating a gradual recovery of bulk pH (Gerardi, 2003). This buffering effect is particularly significant in food waste digestion, where rapid acidification is a common occurrence due to its high biodegradability (Feng et al., 2021).

The bottom digested sludge layer typically exhibits reduced metabolic activity, indicative of substrate depletion and stabilization (Gerardi, 2003). This spatial distribution of metabolic processes demonstrates that, in the absence of mixing, anaerobic digesters tend to develop

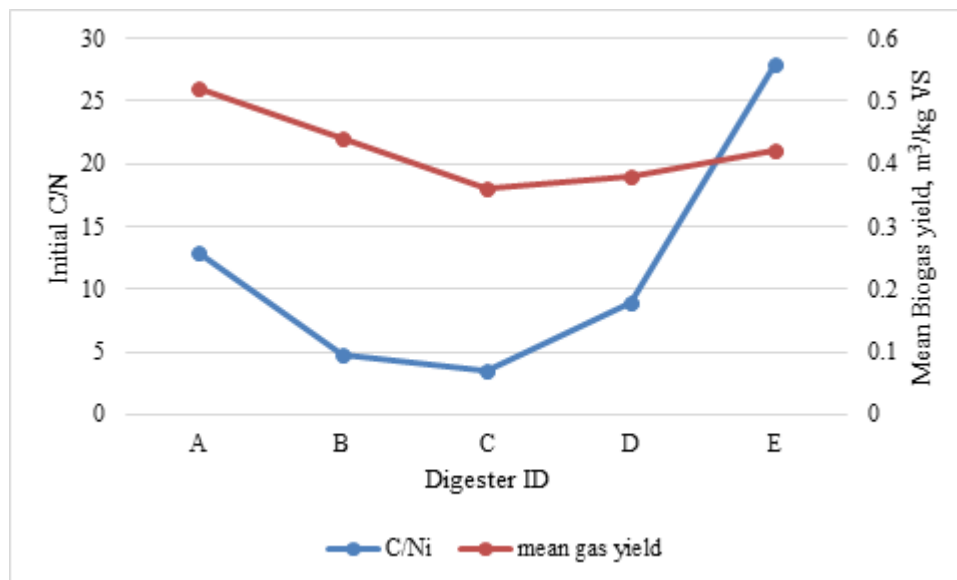


Figure 3: Relation Between Initial C/N Ratio and Cumulative Biogas Yield in U-SPAD System with Varying Mix-Ratio of BSE and TW.

Degradation of proteins in the substrate also releases ammonia ($\text{NH}_3/\text{NH}_4^+$). High ammonia formation from nitrogen in BSE and protein degradation results in ammonia toxicity, however, ammonia toxicity could be mitigated by trace elements. Aquaculture effluents are good sources of trace elements including Co, Cu, Fe, Mn, Ni and Zn (Famoofo and Adeniyi, 2020, Mannzhi et al., 2021). Fe supports iron-mediated anaerobic ammonium oxidation, reducing ammonium accumulation and improving system resilience (Han et al., 2026). Also Co, Fe, and Ni have been identified alongside Se and Mo as trace elements that improves VFA removal and stabilizes digesters during inhibition conditions by improving enzymatic activities and shaping microbial community and metabolic pathways (Afronze et al. 2022, Salazar-Batres and Moreno-Andrade, 2025).

The results suggest that while methanogens are indeed sensitive to low pH, the inhibition is not necessarily irreversible. As VFAs are consumed and buffering capacity improves, methanogenic populations can often regain their activity especially in biofilms and dense flocs enriched with more resilient taxa, such as *Methanosarcina* that are common in anaerobic digestion of food waste and that can utilize H_2/CO_2 and C-1 compounds (Akinsola et al., 2025, Cao et al., 2025) This is demonstrated in Figure 4 as biogas production fluctuated in a U-SPAD all through the 30 days.

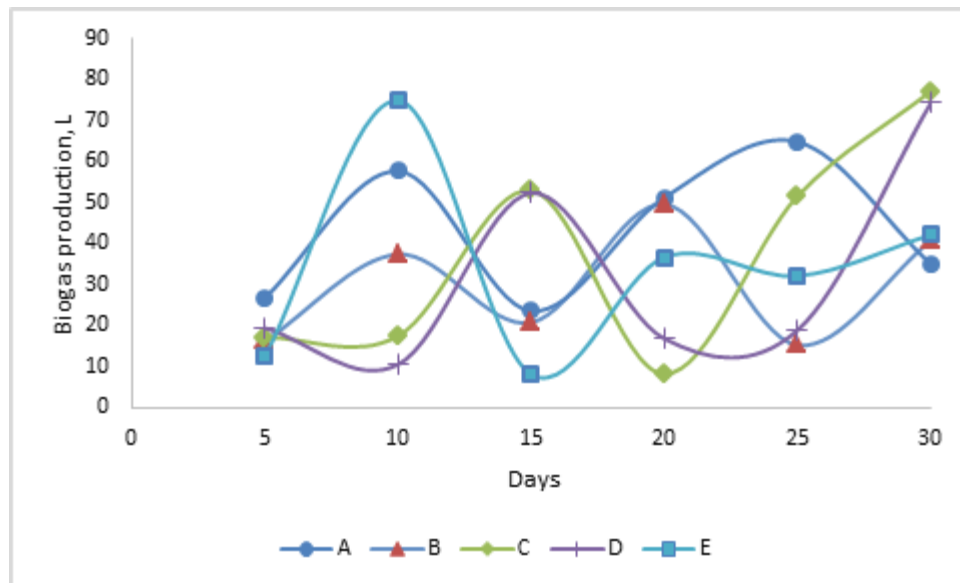


Figure 4: Biogas Production With Reversible Inhibition.

3.2 Effect of Brood Stock Effluent on Biogas Composition

Table 6 shows the percentage composition of the biogas produced from the different digesters revealing that the biogas produced had high methane content (70.4 - 85.5 %) similar to Oduor et al, (2022) but higher than 50-60% reported by Qian et al (2025). Figure 5 shows the impact of the mixed brood stock effluent (BSE)/tap water (TW) ratio on the calorific value of generated biogas. It is noteworthy that, the trend in calorific values of the biogas $B > D > C > A > E$ was coherent with the methane content $B > D > C > A > E$ but was not coherent with the trend in biogas yield means $A > B > E > D > C$.

Table 6: Percentage composition of the biogas produced in the digester.

Component	Percentage Composition (%)				
	A	B	C	D	E
Hydrogen	3.74	2.17	3.21	2.63	4.95
Methane	70.36	85.48	76.85	80.04	64.62
Carbon monoxide	2.24	0.87	1.32	1.02	5.43
Carbon dioxide	4.19	2.02	3.14	2.45	3.18
Hydrogen sulphide	4.56	2.16	3.23	2.76	4.79
Water vapour	5.1	3.64	4.47	4.11	5.54
Nitrogen	6.64	4.36	5.36	4.8	7.47
Oxygen	3.17	1.85	2.42	2.19	3.83

Among the digesters, digesters B, C, and D with mix ratio B75%:T25%, B50%: T50% and B50%: T50% respectively produced biogas within the standard calorific value (20–29 MJ/kg) for common biogas mix and enriched biogas (Deublein & Steinhauser, 2008, Ryckebosch *et al*, 2011) making them the most energy efficient combinations. However, the calorific values

of digesters A and E are similar to typical landfill biogas of 15 – 18 MJ/kg (Deublein & Steinhauser, 2008). Small presence of non-combustible and difficult to burn gases like carbon dioxide (CO₂), nitrogen (N₂), carbon monoxide (CO), hydrogen sulfide (H₂S), and water vapour (H₂O) significantly decreases biogas energy content (Deublein & Steinhauser, 2008). The ideal range of water vapour and nitrogen in raw biogas are 1-5% and 0-15%, respectively and a direct linear relationship exists between non-combustible gas levels and biogas heating value (Sahin et al, 2020; Tomczak et al, 2024). This explains the low gas energy content for digesters A and E with water content of ≥ 5% and nitrogen content > 6.5%.

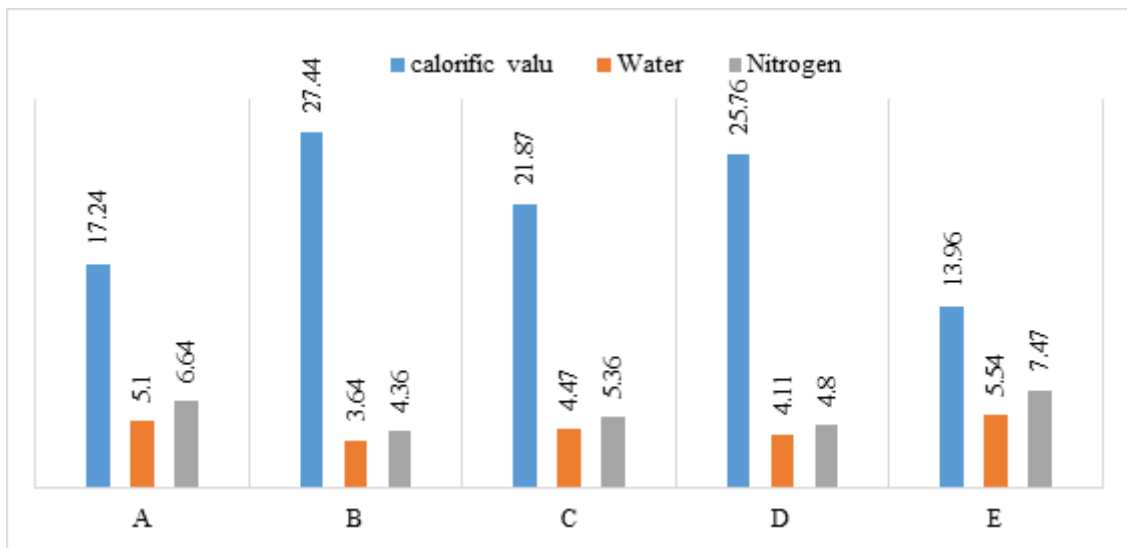


Figure 5: Effect of mixed brood stock effluent (BSE)/tap water (TW) ratio on the calorific value of generated biogas.

1. CONCLUSION

The study demonstrated the systematic replacement of freshwater with aquaculture effluent in an unstirred single-phase anaerobic digestion (U-SPAD) system, reducing fresh water consumption while enhancing methane yield and energy recovery. Biogas was consistently produced and the cumulative yield was appreciable across all mix-ratios of brood stock effluent (BSE) and tap water (TW). The cumulative biogas yield obtained was different for the varying mix-ratios but without any statistical significance. The methane compositions of the various mix-ratio were high (> 70%) and the calorific values were within common biogas and landfill biogas range. In addition, the study reflected that the U-SPAD functioned as self-induced two-phase anaerobic digestion system. The system underscores the fact that system performance is governed by the interplay of spatial heterogeneity substrate characteristics and microbial adaptability. The important implication of the use of U-SPAD under unadjusted

acidic pH and mesophilic conditions is the development of simplified, low-energy reactor configuration for food waste and aquaculture effluent treatment.

AUTHOR CONTRIBUTION

This study was a collaboration among both authors. Authors E. Ukoha-Onuoha provided theoretical framework and guidance for data interpretation. Author F. N. Udenze carried out all experimental works and wrote the draft manuscript. Author E. Ukoha-Onuoha prepared manuscript. The final version of the manuscript was approved by both authors.

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