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Stabilizing High-Load Food Waste Digesters: Bioaugmentation versus pH Adjustment Effects on Microbial Consortium Dynamics and Methanogenesis

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Abstract. *High organic loading of food waste digesters often causes volatile fatty acid (VFA) accumulation, ammonia inhibition, and process failure. This study compared pH adjustment with bioaugmentation using a rumen-derived syntrophic consortium (FUD-BAC1) for stabilizing high-load food waste digesters, emphasizing microbial community dynamics and methane production. Three identical mesophilic (37 °C) continuous stirred-tank reactors (4.0 L working volume) were run in parallel, one reactor per treatment: an unamended control (R_Ctrl), a pH-adjusted reactor (R_pH; $NaHCO_3$ or NaOH added when pH fell below 6.5), and a bioaugmented reactor (R_Bio; single 10% v/v dose of FUD-BAC1). Biogas, methane, VFAs, total ammonia nitrogen (TAN), and culturable microbial groups were monitored for 27 days. The control produced minimal biogas (2.7 ± 0.8 L) and no measurable methane. R_pH produced 9.4 ± 1.2 L of biogas (43.6 ± 2.1% CH_4; 8.5 ± 1.2 L CH_4/kg VS), whereas R_Bio produced 19.4 ± 1.5 L (60.8 ± 2.5% CH_4; 23.1 ± 1.8 L CH_4/kg VS) and achieved the highest substrate degradation (79.2 ± 2.8%, versus 49.0 ± 3.2% for R_pH and 6.4 ± 1.5% for the control). Relative to R_pH, the specific methane yield of R_Bio was about 2.7 times higher. Final total VFA was 3,900, 2,650, and 1,800 mg/L, and final TAN was 2,800, 2,100, and 1,950 mg/L, in the control, R_pH, and R_Bio, respectively. Bioaugmentation was accompanied by higher counts of culturable anaerobic bacteria and methanogens, and by the isolation of presumptive syntrophic acetogens. Under the conditions tested, rumen-derived bioaugmentation was associated with more effective restoration of methanogenesis than pH adjustment alone; replicated, longer-term trials are needed to confirm these findings. This work relates to SDG 7 (Affordable and Clean Energy) and SDG 12 (Responsible Consumption and Production).*

Keywords: *Ammonia Inhibition; Anaerobic Digestion; Bioaugmentation; Food Waste; Methanogenesis; Rumen Inoculum; Syntrophy; Volatile Fatty Acids*

1. Introduction

The global food waste crisis is one of the most serious environmental issues of the twenty-first century. The total amount of food wasted along the food supply chain, from production to consumption, is around 1.3 billion tonnes annually, nearly one-third of all food produced for human consumption (Banks et al., 2018; Uddin et al., 2021). This vast amount of organic waste is a significant environmental risk, as it can generate greenhouse gas emissions during decomposition in landfills and leachate that pollutes water bodies, and it represents a loss of valuable resources invested in food production (Morales-Polo et al., 2018; Zhang et al., 2019). Rapid urbanization has contributed to the surge of waste generation in developing countries such as Nigeria, where inadequate management infrastructure causes

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environmental degradation and prevents the recovery of resources from waste (Adam et al., 2020; Amoo et al., 2024). In this context, anaerobic digestion has become a promising technology, serving several sustainability goals such as reducing organic waste in landfills, creating renewable energy, generating nutrient-rich digestate, and supporting the principles of a circular economy (Anukam et al., 2019; Li et al., 2022; Amoo & Adeleye, 2026; Isyaku et al., 2026).

Anaerobic digestion is a complex biological process consisting of four stages, namely hydrolysis, acidogenesis, acetogenesis, and methanogenesis, which are performed in a syntrophic manner by different microbial communities (Schnürer & Jarvis, 2010; Shah et al., 2014; Amoo et al., 2023). Pre-treatment advances have been reviewed as a means of overcoming hydrolysis-related barriers in biogas production (Amoo et al., 2023). The high organic matter content and biodegradability make food waste an attractive feedstock; however, these same properties make it difficult to digest stably at the high organic loading rates (OLRs) typical of commercial applications (>2.0 g VS/L/day) (Micolucci et al., 2018; Nathia-Neves et al., 2018; Amoo et al., 2023; Amoo, 2026). In addition, the rapid degradation of readily degradable carbohydrates leads to the formation of volatile fatty acids (VFAs), which lower the pH and consequently inhibit methanogenic activity, a condition known as acidification (Chen & Wu, 2019; Gao et al., 2020). Moreover, the degradation of protein-rich components releases ammonia, and when total ammonia nitrogen (TAN) reaches 1,500–3,000 mg/L, methanogenic activity is significantly inhibited, especially that of acetoclastic methanogens (Wang et al., 2016; Ye et al., 2018). This imbalance between acidogenic and methanogenic activity creates a feedback loop of inhibition and further VFA accumulation, which can ultimately result in process failure if not managed properly (Mao et al., 2015; Nsair et al., 2020).

Among the various strategies employed to stabilize high-load anaerobic digesters, pH adjustment through alkalinity addition (sodium bicarbonate, lime, or potassium hydroxide) has been widely practiced because of its simplicity and immediate effect (Montanes et al., 2014; Stavropoulos et al., 2016). The optimum pH range for digester operation, 6.8–7.5, provides a favorable environment for methanogenic activity and shifts the equilibrium between ammonium (NH_4^+) and free ammonia (NH_3) towards NH_4^+, which is less toxic to microbes (Nsair et al., 2020; Wang et al., 2016). However, adjusting the pH does not correct microbial imbalances; it is a reactive rather than a proactive measure (Anukam et al., 2019; Caruso et al., 2019). In contrast, the introduction of exogenous microbial consortia (bioaugmentation) as a proactive stabilization approach has been receiving attention (Kovács et al., 2013; Owamah, 2019). The rumen microbial community is especially promising, with a wide variety of hydrolytic bacteria, including members of the genera *Bacteroides*, *Clostridium*, and *Ruminococcus*; fermentative bacteria, such as *Enterobacter* and *Lactobacillus*; syntrophic acetogens, such as *Syntrophomonas* and *Syntrophobacter*; and methanogenic archaea, such as *Methanobacterium* and *Methanosaeta*, that can degrade complex organic matter efficiently (Castillo-González et al., 2014; Manyi-Loh et al., 2013; Ogbonna et al., 2018). Consortia derived from the rumen are thought to improve the hydrolysis of recalcitrant substrates, increase the diversity of VFA-degrading microbes, and provide robust microbes that tolerate high levels of ammonia and VFAs (Jin et al., 2018; Tambuwal et al., 2019).

Although both approaches have shown potential, there is still a large knowledge gap on their comparative efficacy and mechanisms of action under standardized conditions

(Anukam et al., 2019; Caruso et al., 2019). Few studies have examined how microbial communities change to produce a stabilizing effect, including changes in the abundance of key functional groups and the establishment of syntrophic relationships that support stable methane production (Lim et al., 2020; Treu et al., 2016). An integrated assessment of process performance and microbial community change is therefore needed to understand how different stabilization methods act (Barua & Dhar, 2017; Kumar et al., 2011). In developing countries such as Nigeria, food waste generation is escalating, rumen contents are readily available from abattoirs, and affordable solutions are urgently required (Adam et al., 2020; Amoo et al., 2024). Therefore, the present study was designed to compare pH adjustment and bioaugmentation with a rumen-derived microbial consortium as stabilization strategies for high-load food waste digesters, with emphasis on microbial consortium dynamics and methanogenesis performance, as a step towards an optimized operational protocol for sustainable waste-to-energy conversion.

By converting food waste into methane-rich biogas, anaerobic digestion directly supports Sustainable Development Goal (SDG) 7 (Affordable and Clean Energy), through the generation of renewable energy, and SDG 12 (Responsible Consumption and Production), through the reduction and valorization of food waste. Evaluating low-cost strategies for stabilizing overloaded digesters therefore addresses a practical barrier to these goals.

2. Methods

2.1. Substrate Collection and Preparation

Fruit peels (orange, watermelon and pineapple peels), vegetable scraps and leftover food items were collected as food waste (FW) from markets in Dutse, Jigawa State, Nigeria (Figure 1). To obtain a uniform particle size (<2 mm), the waste was homogenized with an electric blender and stored at 4 °C until use. Rumen liquid content (RLC) was obtained from the Dutse Central Abattoir (Figure 1) and served as the source of the bioaugmentation consortium. The seed inoculum was obtained from an active mesophilic anaerobic digester treating cattle manure, which provided a diverse and metabolically active microbial population.

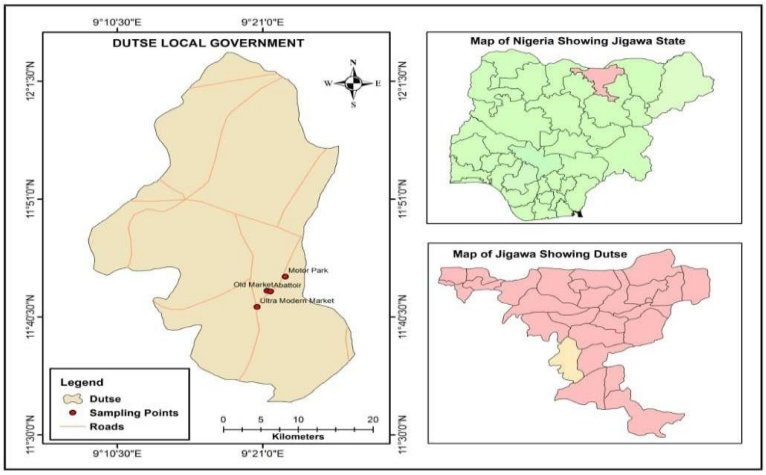

Figure 1. Map of the study area showing Dutse Local Government Area, Jigawa State, Nigeria.

2.2. Substrate Characterization

Physicochemical properties of the food waste and the seed inoculum were measured according to standard methods. Total solids (TS) and volatile solids (VS) were determined gravimetrically after drying at 105 °C and combustion at 550 °C, respectively (American Public Health Association [APHA], 2005). pH was measured with a digital pH meter. Total organic carbon (TOC) was measured by the Walkley–Black dichromate oxidation method, and total Kjeldahl nitrogen (TKN) by the Kjeldahl digestion method. The carbon-to-nitrogen (C/N) ratio was calculated as TOC divided by TKN (APHA, 2005). The characteristics of the food waste and the seed inoculum are shown in Table 1.

Table 1. Physicochemical Characteristics of Food Waste and Seed Inoculum

Parameter	Food Waste (FW)	Seed Inoculum
Total Solids (TS, %)	18.52 ± 0.45	3.24 ± 0.12
Volatile Solids (VS, % of TS)	89.40 ± 1.12	64.15 ± 0.85
pH	5.12 ± 0.10	7.35 ± 0.05
Total Organic Carbon (TOC, g/kg)	420.30 ± 5.10	21.40 ± 0.60
Total Kjeldahl Nitrogen (TKN, g/kg)	18.25 ± 0.35	2.15 ± 0.08
C/N Ratio	23.03 ± 0.50	9.95 ± 0.20

2.3. Bioaugmentation Consortium Preparation

The bioaugmentation consortium was enriched and cultured in the Environmental Sciences and Microbiology Laboratory, Federal University Dutse. It was isolated from rumen fluid collected from the local slaughterhouse according to the method of Amoo et al. (2025). The enrichment medium was anaerobic basal medium supplemented with 2.0 g/L of propionate and acetate as sole carbon sources and incubated at 37 ± 1 °C. After five successive transfers under strictly anaerobic conditions, the consortium formed a stable syntrophic community, composed primarily of *Syntrophobacter* and *Smithella* spp. Methanogens (including the acetoclastic and hydrogenotrophic *Methanosarcina barkeri*) were present together with syntrophic propionate-oxidizing bacteria. Before inoculation, the consortium was adjusted to approximately 1.2×10^{10} cells/mL. The composition of the FUD-BAC1 consortium is given in Table 2.

Table 2. Microbial Composition of FUD-BAC1 Bioaugmentation Consortium

Microbial Group	Count (cells/mL)
Syntrophic Propionate-Oxidizing Bacteria	4.8×10^9
Acetoclastic Methanogens (*Methanosarcina* spp.)	3.6×10^9
Hydrogenotrophic Methanogens	2.1×10^9
Fermentative Bacteria	1.5×10^9

2.4. Experimental Design and Reactor Setup

Three identical continuous stirred-tank reactors (CSTRs), each with a total volume of 5.0 L and a working volume of 4.0 L, were operated in parallel at 37 ± 0.5 °C. Each reactor received a different stabilization treatment to assess its effectiveness in high-load food waste digesters (Amoo, 2025). Reactor 1 (R_Ctrl) served as the unamended control and received no bioaugmentation or chemical treatment throughout the operational period; it provided a

baseline for natural process failure and self-recovery under organic loading stress. Reactor 2 (R_pH) received pH adjustment only. Reactor 3 (R_Bio) received a single dose (10% v/v of the active reactor volume) of the FUD-BAC1 consortium to test its ability to restore process stability by introducing syntrophic propionate-oxidizing bacteria and methanogenic archaea.

Operating phases. The 27-day experiment comprised two phases applied to all three reactors. In Phase I (OLR escalation), the reactors were started at an organic loading rate (OLR) of 2.0 g VS/L/day (hydraulic retention time, HRT = 20 days) for 7 days, then increased stepwise to 4.0 g VS/L/day for 7 days and to 6.0 g VS/L/day for 6 days, to determine loading limits and to induce acidification and methanogenic failure, consistent with operational parameter ranges reviewed by Amoo et al. (2023). Phase II (recovery) began on day 20, when process failure was indicated by a pH below 6.5, total VFA above 3,000 mg/L, and methane content below 10% in all three reactors. From day 20, the OLR was held at 3.0 g VS/L/day for the remaining 7 days, and the recovery interventions began: R_pH received pH adjustment, R_Bio received the single dose of FUD-BAC1 on day 20, and R_Ctrl received no intervention and continued to be fed at 3.0 g VS/L/day throughout Phase II.

pH control (R_pH). The pH was measured daily with a calibrated pH meter. When the pH fell below 6.5, 1.0 M $NaHCO_3$ was added first to provide buffering capacity, and 1.0 M NaOH was added subsequently when rapid correction was required. Each addition was 10 mL, applied until the target range of 7.0–7.2 was reached. The pH was re-measured 30 minutes after each addition and checked twice daily between additions to maintain the target range. In total, 12 additions (120 mL) were made between day 20 and day 27. R_Ctrl and R_Bio were not pH-adjusted.

2.5. Analytical Methods

2.5.1. Biogas Production and Composition

Daily biogas production was measured by the water displacement method with an inline CO_2 scrubbing trap (3.0 M NaOH solution) to quantify methane. The composition of the biogas (CH_4 and CO_2) was determined by gas chromatography (Agilent 7890B) with a thermal conductivity detector (TCD). Methane content was calculated as shown in Equation (1):

$$CH_4(\%) = \frac{Volume\ CH_4}{Total\ biogas\ volume} \times 100 \ldots\ldots\ldots eqn.\ 1$$

2.5.2. Volatile Fatty Acid Analysis

Volatile fatty acids (VFAs), namely acetic, propionic and butyric acids, were measured by high-performance liquid chromatography (HPLC, Shimadzu LC-20AD) with a UV–Vis detector, as described in APHA (2005). Samples were centrifuged at 10,000 rpm for 10 min and then passed through 0.45 µm membrane filters before analysis on a C18 column, with a mobile phase of 25 mM phosphate buffer (pH 2.5) at a flow rate of 1.0 mL/min.

2.5.3. Alkalinity and Ammonia Analysis

Total alkalinity (TA) and partial alkalinity (PA) were determined by standard acid titration to pH 4.3 and 5.75, respectively (Schnürer & Jarvis, 2010). Total ammonia nitrogen (TAN) was measured by the Nessler method with spectrophotometric measurement at 425 nm (APHA, 2005). Free ammonia nitrogen was not measured, and no nitrogen mass balance was determined.

2.5.4. Microbial Community Analysis

Digestate samples (15 mL) were collected from all three reactors during the stable, acidified, and recovery phases for evaluation of microbial dynamics. Strict anaerobes were counted by the agar roll-tube method (Hungate, 1969; Ogbonna et al., 2018) and facultative anaerobes by the most probable number (MPN) method (Ogbonna et al., 2018); counts are expressed as CFU/g. Acetoclastic and hydrogenotrophic methanogens were enumerated by the MPN method using acetate (50 mM) and H_2/CO_2 (80:20, v/v) as substrates, respectively, with methanogenic growth confirmed by methane production measured by gas chromatography.

Identification of bacterial genera (Table 4). Bacterial isolates from the bioaugmented reactor (R_Bio) were purified by repeated streaking and tentatively assigned to genera on the basis of colony and cell morphology, Gram reaction, and catalase, oxidase, and fermentation profile tests, by comparison with descriptions in Bergey's Manual (Whitman, 2015). No 16S rRNA gene sequencing or other molecular identification was performed, so all genus-level assignments are presumptive.

Limitations of culture-based characterization. Only a small fraction of the microorganisms in anaerobic digesters can be cultivated with standard media (Amann et al., 1995; Staley & Konopka, 1985). Strict anaerobes, syntrophs, and methanogens are particularly difficult to isolate and enumerate (Kumar et al., 2011), and the media and incubation conditions select for particular taxa. Counts therefore describe the culturable fraction only and may not reflect relative abundance or activity in situ. Morphology- and biochemistry-based identification is also less resolved than sequence-based identification.

2.6. Statistical Analysis

All data are presented as mean ± standard deviation (SD) of triplicate analytical measurements made on a single reactor per treatment. These are technical (analytical) replicates, not independent reactor replicates. Because each treatment was applied to only one reactor, treatment is confounded with reactor identity, and treating technical replicates as independent units would be pseudoreplication (Hurlbert, 1984). Accordingly, one-way analysis of variance (ANOVA) with Tukey's honestly significant difference (HSD) post hoc test ($p < 0.05$) in SPSS version 25 was used only to describe whether measurements differed by more than analytical variability, and the resulting p-values are not interpreted as evidence of treatment-level effects. Pearson's correlation coefficients were calculated for exploratory purposes only (n = 27 paired observations), and are not interpreted as evidence of causation.

3. Results and Discussion

3.1. Biogas Production and Methane Yield

The stabilization strategies differed clearly in biogas and methane output over the 27 days (Figure 2). The control reactor (R_Ctrl) produced minimal biogas (2.7 ± 0.8 L) and no measurable methane, suggesting process failure. The pH-adjusted reactor (R_pH) generated 9.4 ± 1.2 L of biogas with a methane content of 43.6 ± 2.1% (methane yield: 8.5 ± 1.2 L CH_4/kg VS). The bioaugmented reactor (R_Bio) had the highest output, with 19.4 ± 1.5 L of biogas, a methane content of 60.8 ± 2.5%, and a methane yield of 23.1 ± 1.8 L CH_4/kg VS. Substrate degradation followed the same order: control (6.4 ± 1.5%), R_pH (49.0 ± 3.2%), and R_Bio (79.2

± 2.8%). Because each treatment was a single reactor and the ± values describe analytical replicates, these are observed differences between reactors, not statistically established treatment effects. Comparison basis: because no methane was measurable in R_Ctrl, percentage increases relative to the control are undefined and are not reported. All relative comparisons are made between R_Bio and R_pH, the two reactors that produced methane. Relative to R_pH, R_Bio had a specific methane yield that was 14.6 L CH_4/kg VS higher (23.1 versus 8.5 L CH_4/kg VS, about 2.7 times or 172% higher), a methane content that was 17.2 percentage points higher (60.8% versus 43.6%), substrate degradation that was 30.2 percentage points higher (79.2% versus 49.0%; 62% higher in relative terms), and about twice the biogas volume (19.4 versus 9.4 L). The larger output of R_Bio is consistent with the idea that the added consortium supplied microorganisms able to carry out all four steps of anaerobic digestion (Schnürer & Jarvis, 2010; Shah et al., 2014), but this explanation was not tested directly.

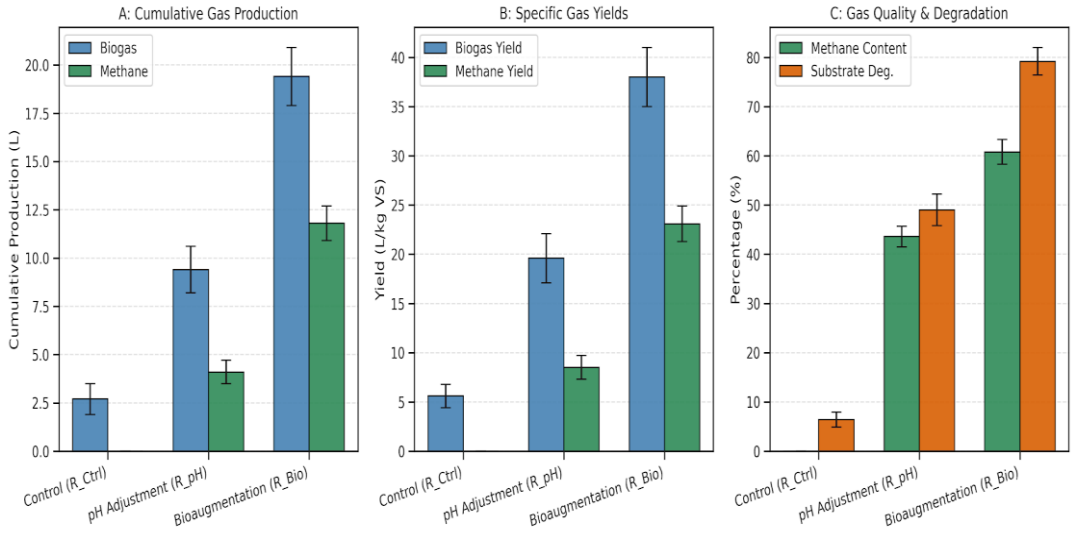

Figure 2. Anaerobic digestion performance across treatments: (a) cumulative biogas and methane production; (b) specific biogas and methane yields; (c) methane content and substrate degradation.

The methane yield of R_Bio (23.1 L CH_4/kg VS) was comparable to or higher than values reported in some other studies. Jin et al. (2018) reported 18.5 L/kg VS for corn straw without rumen inoculation, Tambuwal et al. (2019) 12.5 L/kg VS for mono-digestion of rumen content, Gao et al. (2020) 15.2 L/kg VS for food waste with yeast addition, and Wang et al. (2020) 14.8 L/kg VS under pH control. Differences in substrate, inoculum, loading, and operating conditions limit such comparisons. The conditions in this study, the recovery-phase OLR (3.0 g VS/L/day), the diverse microbial consortium in the rumen inoculum, the substrate composition (50% FW, 50% H_2O), and the mesophilic temperature (37 ± 0.5 °C), may have contributed to the yield.

3.2. Volatile Fatty Acid Dynamics

VFA accumulation was an important indicator of process stability across treatments. In the control reactor, total VFA reached 3,900 ± 330 mg/L by day 27, dominated by acetic acid (2,100 ± 180 mg/L), with propionic acid (1,100 ± 90 mg/L) and butyric acid (700 ± 60 mg/L) also at high levels (Figure 3). The pH-adjusted reactor had lower VFA levels, with a

total of 2,650 ± 230 mg/L (including 1,400 ± 120 mg/L acetic acid, 800 ± 70 mg/L propionic acid, and 450 ± 40 mg/L butyric acid). The bioaugmented reactor had the lowest VFA concentrations, with a total of 1,800 ± 155 mg/L, which is 54% lower than the control and 32% lower than R_pH. The higher propionate levels in the control and R_pH suggest that syntrophic propionate oxidation was not fully established in those reactors, whereas the lower propionate level in R_Bio is consistent with the establishment of propionate-oxidizing syntrophic populations introduced with FUD-BAC1.

Volatile fatty acids are key indicators of process instability in anaerobic digesters (Mao et al., 2015; Nsair et al., 2020). As shown in Figure 3, the total VFA concentration in the control reached 3,900 mg/L, exceeding the concentration range (2,000–3,000 mg/L) reported to inhibit methanogens (Micolucci et al., 2018; Nathia-Neves et al., 2018). Propionate concentrations were high in the control reactor (1,100 mg/L) and in R_pH (800 mg/L), indicating incomplete syntrophic oxidation. Propionate is a particularly problematic substrate because its oxidation is thermodynamically unfavorable, and syntrophic cooperation with hydrogenotrophic methanogens is needed to keep the H_2 partial pressure low (Barua & Dhar, 2017; Shah et al., 2014). The lower propionate level in R_Bio (600 mg/L) suggests that propionate-oxidizing syntrophic communities were established through the FUD-BAC1 consortium.

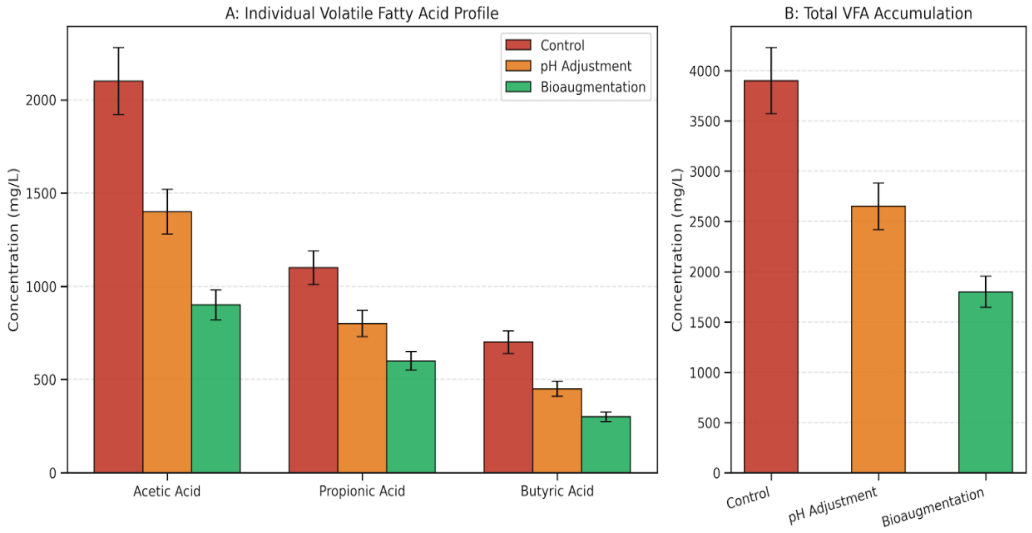

Figure 3. Volatile fatty acid (VFA) concentrations after 27 days: (a) individual VFAs (acetic, propionic, and butyric acids) and (b) total VFA in the control (R_Ctrl), pH-adjusted (R_pH), and bioaugmented (R_Bio) reactors.

The 54% reduction in total VFA in R_Bio relative to the control (from 3,900 to 1,800 mg/L; Figure 3) may be explained by three possible, complementary mechanisms, which were not tested individually. First, the introduction of syntrophic acetogenic bacteria such as *Syntrophomonas* sp. and *Syntrophobacter* sp. may have improved the conversion of propionate and butyrate to acetate and hydrogen, minimizing VFA accumulation (Manyi-Loh et al., 2013; Shah et al., 2014). Second, higher methanogenic activity, specifically of acetoclastic methanogens (from not detected to 3.4×10^5 CFU/g), may have promoted the conversion of acetate to methane (Kumar et al., 2011; Lim et al., 2020). Third, hydrogenotrophic methanogens (*Methanobacterium* sp.) may have kept the hydrogen partial pressure low, favoring thermodynamically favorable VFA degradation (Barua & Dhar, 2017; Treu et al.,

2016). R_pH showed lower VFA concentrations than the control, possibly because pH adjustment created more favorable conditions for methanogenic activity; however, its residual VFA concentrations remained higher than those of R_Bio, which may reflect the fact that pH adjustment did not directly address the microbial imbalance.

3.3. Ammonia Dynamics

TAN increased gradually in all reactors during the 27-day operation, consistent with the degradation of protein in the food waste. The initial TAN concentrations were equal (1,200 ± 80 mg/L). In the control reactor, TAN reached 2,400 ± 120 mg/L by day 14, above the concentration range (1,500–2,000 mg/L) at which methanogenesis may be inhibited. The pH-adjusted reactor showed slower accumulation, reaching 2,000 ± 110 mg/L by day 14 and 2,100 ± 115 mg/L by day 27 (Figure 4). The bioaugmented reactor had the lowest TAN concentrations throughout, with a final value of 1,950 ± 100 mg/L on day 27, which is 30% lower than that of the control (2,800 ± 150 mg/L) and 7% lower than that of R_pH (Figure 4). One possible explanation for the lower TAN in R_Bio is greater microbial assimilation of ammonia into biomass by the diverse and metabolically active FUD-BAC1 consortium. This is a hypothesis only: no nitrogen mass balance and no free ammonia measurements were made, so the mechanism cannot be confirmed from the present data.

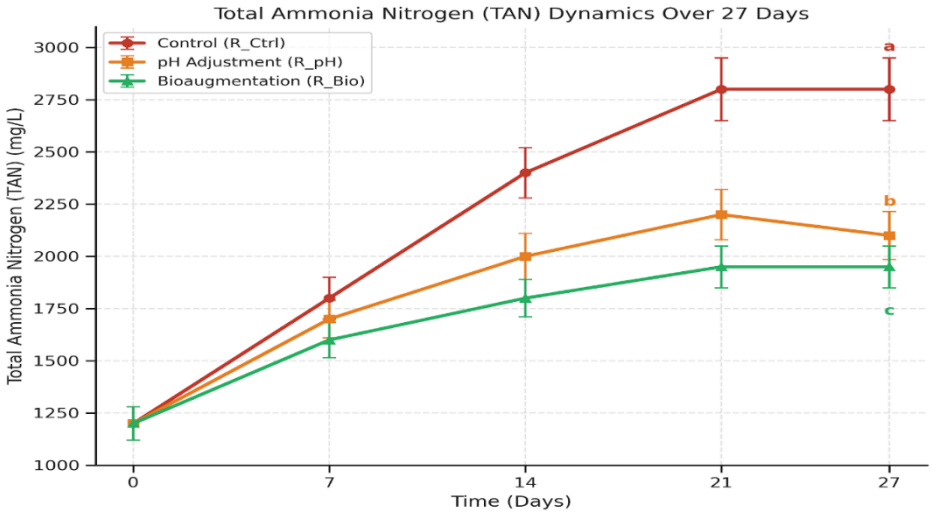

Figure 4. Total ammonia nitrogen (TAN) dynamics over 27 days in the control (R_Ctrl), pH-adjusted (R_pH), and bioaugmented (R_Bio) reactors

TAN in the control reactor reached 2,800 mg/L (Figure 4), which is within the range (1,500–3,000 mg/L) reported to inhibit methanogenic activity (Nsair et al., 2020; Ye et al., 2018). The lower TAN in R_Bio (1,950 versus 2,800 mg/L in the control) may be explained by two hypotheses that are not mutually exclusive. First, the introduced consortium may have assimilated ammonia into microbial biomass, leaving less ammonia in solution (Castillo-González et al., 2014; Ogbonna et al., 2018). Second, reduced VFA accumulation may have kept the pH closer to the optimum, lowering the proportion of TAN present as toxic free ammonia (NH_3) (Rajagopal et al., 2013; Schnürer & Jarvis, 2010; Wang et al., 2016). Neither hypothesis was tested, because no nitrogen mass balance and no free ammonia measurements were made. In R_pH, pH adjustment maintained conditions suitable for methanogens without

lowering total ammonia (Montanes et al., 2014; Stavropoulos et al., 2016), which is consistent with pH adjustment being a reactive rather than a proactive measure. TAN and methane yield were negatively correlated ($r = -0.784$), which is consistent with, but does not prove, an inhibitory effect of ammonia on methanogenic activity. Free ammonia (NH_3) is known to diffuse passively across cell membranes, causing proton imbalance and potassium depletion that may inhibit methanogenic enzymes (Wang et al., 2016; Ye et al., 2018).

3.4. Microbial Community Dynamics

The culturable microbial groups differed among the three reactors (Table 3). Facultative anaerobic bacteria (6.7×10^5 CFU/g) and strict anaerobic bacteria (11.4×10^5 CFU/g) were detected initially, whereas methanogenic archaea were not detected. In the control reactor, facultative anaerobes declined by 79% (from 6.7×10^5 to 1.4×10^5 CFU/g) and strict anaerobes by 75% (from 11.4×10^5 to 2.8×10^5 CFU/g), and methanogens remained undetected (Table 3). In R_pH, counts were intermediate: 4.8×10^5 CFU/g for facultative anaerobes, 7.2×10^5 CFU/g for strict anaerobes, and 1.2×10^5 and 0.8×10^5 CFU/g for acetoclastic and hydrogenotrophic methanogens, respectively. R_Bio had the highest counts: facultative anaerobes increased by 33% (to 8.9×10^5 CFU/g) and strict anaerobes by 47% (to 16.8×10^5 CFU/g), while acetoclastic and hydrogenotrophic methanogens rose from not detected to 3.4×10^5 and 2.1×10^5 CFU/g, respectively (Table 3). Percentage increases are not given for methanogens because their initial counts were below the detection limit. Methane production was positively correlated with acetoclastic methanogen counts ($r = 0.912$), which is consistent with a key role for these organisms in process recovery.

In the control reactor, methanogens were not detected during the 27 days, and facultative and strict anaerobic bacteria decreased by 79% and 75%, respectively (Table 3). This reduction may have been caused by VFA accumulation, pH decline, and ammonia inhibition (Kumar et al., 2011; Lim et al., 2020), although these causal links were not tested. The failure of the indigenous microbial community under high VFA and ammonia concentrations illustrates the susceptibility of digesters to process instability. In R_pH, microbial populations were partially restored (Table 3). Methanogens were present, indicating partial recovery, but at lower counts than in R_Bio. This suggests that pH adjustment alone may restore conditions for methanogenic activity but not necessarily the microbial diversity and metabolic redundancy that support process stability; diversity was not measured here, so this remains a hypothesis.

Table 3. Culturable Microbial Populations After 27 Days (CFU/g × 10^5). Counts are means of triplicate analytical determinations on a single reactor per treatment.

Microbial Group	Initial	Control	pH Adjustment	Bioaugmentation
Facultative Anaerobic Bacteria	6.7	1.4	4.8	8.9
Strict Anaerobic Bacteria	11.4	2.8	7.2	16.8
Acetoclastic Methanogens	ND	ND	1.2	3.4
Hydrogenotrophic Methanogens	ND	ND	0.8	2.1

ND = not detected (<10^2 CFU/g). Control = R_Ctrl; pH adjustment = R_pH; bioaugmentation = R_Bio.

After bioaugmentation, culturable populations in R_Bio were the highest: facultative anaerobic bacteria were 33% above the initial count, strict anaerobic bacteria 47% above, and acetoclastic and hydrogenotrophic methanogens reached 3.4×10^5 and 2.1×10^5 CFU/g, respectively (Table 3). This suggests enrichment of key functional groups in R_Bio, which may partly explain its better performance. The strong positive correlation between acetoclastic methanogen counts and methane yield ($r = 0.912$) is consistent with the major contribution of acetate-degrading methanogens, which are responsible for about 70% of the CH_4 produced in anaerobic digesters (Kumar et al., 2011; Lim et al., 2020).

3.5. Syntrophic Interactions and Metabolic Pathways

Culture-based characterization of R_Bio indicated a bacterial community comprising several functional groups relevant to anaerobic digestion (Table 4). Genus-level assignments are presumptive. Hydrolytic bacteria (*Bacteroides* sp. and *Ruminococcus* sp.), which break down complex polymers, and fermentative bacteria (*Clostridium* sp., *Enterobacter* sp., *Lactobacillus* sp., *Staphylococcus* sp., and *Streptococcus* sp.), which acidify and produce hydrogen, were isolated. Syntrophic acetogens (*Syntrophomonas* sp. and *Syntrophobacter* sp.) and the acetogen *Acetobacterium* sp. were also recorded (Table 4), which is consistent with propionate and butyrate oxidation and acetate production through syntrophic interactions. These results are consistent with a functional community covering the main stages of anaerobic digestion, including syntrophic VFA oxidation, which is often rate-limiting. However, they do not demonstrate a complete community: the isolates are a culturable subset, were not quantified individually.

Table 4. Presumptive Bacterial Genera Isolated from the Bioaugmented Reactor (R_Bio)

Isolate	Gram Reaction	Morphology	Metabolic Role
Acetobacterium sp.	+	Rod	Acetogenesis
Bacteroides sp.	−	Rod	Hydrolysis
Clostridium sp. (2 species)	+	Rod	Fermentation/Acetogenesis
Enterobacter sp.	−	Rod	Acidogenesis
Lactobacillus sp.	+	Rod	Acidogenesis
Ruminococcus sp.	−	Cocci	Hydrolysis
Staphylococcus sp.	+	Cocci	Acidogenesis
Streptococcus sp.	+	Cocci	Acidogenesis
Syntrophomonas sp.	−	Rod	Syntrophic Acetogenesis
Syntrophobacter sp.	−	Rod	Syntrophic Acetogenesis

As shown in Table 4, Syntrophomonas sp. and Syntrophobacter sp. were identified (presumptively) in R_Bio. Syntrophic interactions between organisms are important for stable methanogenesis (Anukam et al., 2019; Shah et al., 2014), and these organisms can contribute in three ways. First, they can degrade propionate only when syntrophically associated with hydrogenotrophic methanogens, which ensure favorable thermodynamics (Barua & Dhar, 2017). The oxidation of propionate to acetate, carbon dioxide, and hydrogen is thermodynamically unfavorable under standard conditions ($\Delta G^{\circ\prime} = +76$ kJ/mol) but becomes favorable when hydrogenotrophic methanogens maintain a hydrogen partial pressure below 10^{-4} atm. Second, they convert butyrate to acetate and hydrogen, which are then consumed

by methanogens (Gruninger et al., 2014; Manyi-Loh et al., 2013). Third, in partnership with hydrogenotrophic methanogens, they help keep the hydrogen partial pressure low, thus preventing inhibition of syntrophic acetogenesis (Lim et al., 2020; Treu et al., 2016). The lower propionate and butyrate levels and higher methane production in R_Bio are consistent with the establishment of such syntrophic communities, but a causal link was not demonstrated. Acetobacterium sp. was also recorded in R_Bio; acetogens of this genus produce acetate from substrates such as hydrogen and carbon dioxide through the Wood–Ljungdahl pathway (Manyi-Loh et al., 2013).

3.6. Limitations and Future Research

The conclusions of this study were stated based on the limits of its design. (1) Each treatment was run in a single reactor, so the reported standard deviations describe analytical replicates on one reactor, not independent reactor replicates; treatment effects cannot be separated from reactor-to-reactor variation, and no treatment-level significance is claimed (Hurlbert, 1984). (2) The experiment lasted 27 days with a single dose of FUD-BAC1, so it does not show whether the effect persists over several hydraulic retention times or whether repeated dosing would be beneficial. (3) Microbial groups were quantified by culture-based counts, which capture only the culturable fraction of the community, and genus-level identification from isolates is presumptive and less resolved than sequence-based identification. (4) The explanations offered for lower TAN (microbial assimilation and changes in free ammonia) and for lower VFA are hypotheses; neither a nitrogen mass balance nor free ammonia measurements were made. (5) The economic feasibility of rumen-derived bioaugmentation was not assessed. Future work should therefore include replicated reactors, longer operation under controlled organic loading, repeated-dosing trials, measurement or calculation of free ammonia from TAN, pH, and temperature (Anthonisen et al., 1976) together with nitrogen mass balances, culture-independent community analysis (for example, 16S rRNA gene amplicon sequencing) to confirm persistence of the introduced consortium, and a techno-economic evaluation (Amoo & Movlyanov, 2026; Amoo & Adeleye, 2026).

Conclusions

Within the limits of a 27-day, single-dose experiment with one reactor per treatment, bioaugmentation with the rumen-derived FUD-BAC1 consortium was associated with better recovery of high-load food waste digesters than pH adjustment alone. R_Bio reached a specific methane yield of 23.1 L CH_4/kg VS, compared with 8.5 L CH_4/kg VS in R_pH (about 2.7 times higher); the control produced no measurable methane, so no control-based percentage is reported. Total VFA in R_Bio was 54% lower than in the control (from 3,900 to 1,800 mg/L) and 32% lower than in R_pH, and final TAN was lower in R_Bio (1,950 mg/L) than in the control (2,800 mg/L; 30% lower) and R_pH (2,100 mg/L; 7% lower). The lower TAN is a hypothesis that requires nitrogen mass balance and free ammonia data to confirm. Culturable acetoclastic and hydrogenotrophic methanogens increased from not detected to about 10^5 CFU/g in R_Bio, and presumptive hydrolytic, fermentative, and syntrophic acetogenic bacteria were isolated. pH adjustment improved performance relative to the control but left higher residual VFA and lower methane production than bioaugmentation. These results suggest that rumen-derived bioaugmentation is a promising, locally sourced option for stabilizing overloaded food waste digesters, in line with SDG 7 and SDG 12.

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Conflicts of Interest

The authors declare no conflict of interest.

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