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Research Article

Enhancing Methane Production from Food Waste via Anaerobic Digestion Using Waste-Derived Hydrogels: Improved VFA Conversion and Microbial Niche Formation

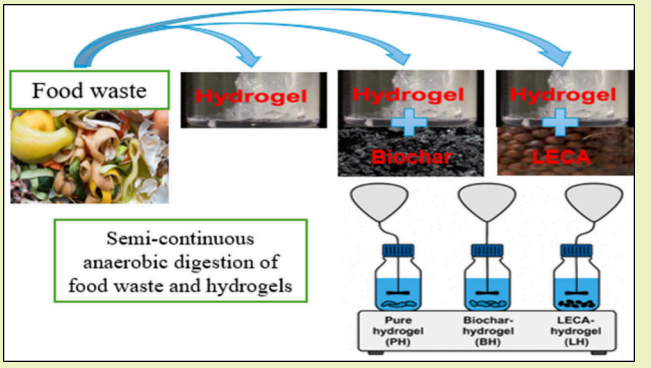
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ABSTRACT: Anaerobic digestion (AD) offers a sustainable approach to food waste valorization through biogas production. Hydrogels, known for high water retention, porosity, and microbial compatibility, are increasingly explored as AD additives to enhance substrate diffusion, pH buffering, and microbial colonization. This study investigates waste-derived hydrogel materials, i.e., pure hydrogel (PH), biochar-hydrogel (BH), and LECA-hydrogel (LH), as multifunctional additives to enhance methane yield under varying organic loading rates (OLRs). At low OLR (0.3 g VS/L/d), BH40 (40 wt % biochar-hydrogel) achieved the highest methane yield (3.71 ± 0.21 L/g VS), producing 27.9% more methane than PH40 (40 wt % hydrogel) due to its buffering and conductive properties that supported syntrophic microbial activity and volatile fatty acids (VFAs) conversion. Conversely, at high OLR (0.9 g VS/L/d), PH40 yielded the highest methane (4.07 ± 0.28 L/g VS), attributed to improved pH stability and VFA utilization. Microbial analysis revealed PH40 enriched key methanogenic taxa, including *Bacilli*, *Synergistia*, and *Cloacimonadia*. Principal component analysis revealed hydrogel additives shaped distinct microbial communities, with PH40 promoting a methanogen-enriched cluster. Overall, this study highlights the novel use of waste-derived hydrogels as dual-function AD enhancers, demonstrating their cost-effective potential to improve methane yield while contributing to circular bioeconomy and sustainable waste-to-energy solutions.



KEYWORDS: *Anaerobic digestion, Food waste, Hydrogel, Biochar, Volatile fatty acids, Methane*

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1. INTRODUCTION

Food waste (FW) is a significant global environmental challenge, constituting a major portion of municipal solid waste in urban areas. According to the Food and Agriculture Organization (FAO), approximately one-third of the world's food is lost across various stages, including production, transportation, distribution, and consumption, amounting to an annual disposal of 130 million tons of FW.[1] If inadequately managed, FW leads to severe environmental impacts, including the emission of greenhouse gases (e.g., methane from landfills), nutrient leaching into water bodies, eutrophication, heavy metal pollution, and excessive algal blooms, which collectively degrade water quality and ecosystem health.[2]

Anaerobic digestion (AD) presents a sustainable solution by stabilizing organic matter and reducing environmental risks while simultaneously producing renewable energy in the form of biogas. AD also minimizes odor, pathogen load, and landfill dependency, contributing to nutrient recycling and circular bioeconomy efforts.[3] Therefore, valorizing FW through AD supports global sustainability goals by turning waste liabilities into resource opportunities. Decentralized AD systems in Singapore have already demonstrated feasibility, reducing waste transport costs while providing renewable energy to local establishments such as hawker centers.[4,5]

Nevertheless, the overall efficiency of methane production in AD largely depends on microbial dynamics across its four sequential stages: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. In the initial stages, hydrolytic and fermentative bacteria break down complex organic matter into simpler monomers and volatile fatty acids (VFAs). Subsequently, acetogenic bacteria convert VFAs into acetate,

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hydrogen, and carbon dioxide, which are the key substrates for methanogenic archaea. Disruptions in the syntrophic interactions between these microbial groups, particularly during VFAs accumulation, can lead to process instability and pH imbalance, ultimately inhibiting methane yield.[6] Therefore, enhancing interspecies electron transfer and stabilizing VFAs conversion are critical strategies for improving AD performance.[7,8]

To enhance AD efficiency, various functional additives have been explored, including biochar,[9] activated carbon,[10] zeolite,[11] and chitosan,[12] each exerting distinct roles in improving microbial activity, electron transfer, and process stability. Biochar's high surface area and porous matrix enhance microbial colonization, buffer pH, and adsorb inhibitors, accelerating VFAs degradation and yielding up to 30% more methane than systems without biochar.[13,14] In addition, its electrically conductive structure facilitates direct interspecies electron transfer (DIET) by serving as an electron conduit between syntrophic bacteria and methanogenic archaea.[15] This physical bridge shortens electron transfer distances, bypass the slower interspecies hydrogen transfer pathway, and accelerates the conversion of VFAs to methane.[16] Next, activated carbon serves as a conductive support that reduces mass transfer resistance and limits intermediate accumulation.[17] For instance, Zhang et al.[18] reported a 41% increase in methane yield in a 1,000 L pilot-scale digester with activated carbon supplementation. Similarly, zeolite, with its strong cation-exchange and adsorption capacity, removes ammonia and other inhibitors, thereby creating a stable environment for methanogenesis.[19] Amen et al.[11] demonstrated that the addition of modified zeolite mixture enhance methane content up to 88%, compared with the control reactor. Chitosan, a biodegradable biopolymer with strong flocculation properties, enhances microbial aggregation, prevents acidification and improves methanogenic activity.[12] In particular, redox-active chitosan and metal oxides can restructure microbial spatial niches, accelerate DIET, and improve methane production efficiency by 35%.[9] Despite these benefits, the economic feasibility, long-term stability, and environmental sustainability of such additives remain key challenges for large-scale AD implementation.

This study presents an innovative approach by repurposing okara-based waste hydrogels from vegetable cultivation residues in Singapore,[20] as cost-effective, sustainable additives for AD. Okara, a soybean-processing byproduct rich in organic matter, is abundantly and often underutilized, making it an attractive feedstock for value-added applications. Repurposing okara-derived hydrogels not only diverts agricultural waste from disposal but also aligns with circular bioeconomy principles by transforming a low-value residue into a functional AD additive. The base hydrogel material, referred to as pure hydrogel (PH), is a chitosan-based substrate with high water retention and microbial aggregation capabilities. Two modified hydrogel variants were used during the vegetable cultivation trials: Biochar-hydrogel (BH), which integrates biochar to enhance electrical conductivity, pH buffering, and DIET; and LECA-hydrogel (LH), which incorporates lightweight expanded clay aggregate (LECA) to improve porosity, surface area, and microbial attachment sites. Unlike traditional powdered or granular additives, these hydrogel composites combine the functional benefits of their embedded materials with the structural stability and reusability of the hydrogel matrix, enabling more uniform distribution in the AD and reducing washout losses.[21] Recovered postharvest, these waste hydrogels were evaluated for their potential to enhance AD performance. Our previous research demonstrated that chitosan-based materials promote the colonization of methanogens, thereby enhancing methane production.[22] They create spatial ecological niches that support syntrophic interactions, promoting microbial synergy and enhancing process resilience. In AD systems, the hydrogel-to-feedstock ratio can influence methane yield by altering the availability of surface area for microbial attachment, the extent of pH buffering, and the degree of inhibitory compound adsorption. Similarly, variations in the organic loading rate (OLR) affect microbial community balance and process stability, with higher OLR increasing the risk of VFAs accumulation and acidification. Understanding the combined effects of hydrogel dosing and OLR is therefore essential for optimizing methane production while maintaining stable operation.[23] However, these parameters remain unexplored for okara-based hydrogel composites, and their potential as functional additives in AD has yet to be realized.

While functional additives offer clear performance benefits, their large-scale application in AD systems faces several challenges, including the high cost or inconsistent availability of certain additive materials, potential leaching of metals or other constituents into the digestate, uncertain long-term stability under continuous operation, and variable effectiveness across different feedstocks or reactor configurations.[24] Operational concerns such as dosing strategies, mixing efficiency, and additive recovery also influence process economics.[25] These challenges highlight the need to develop cost-effective, sustainable, and reusable additive platforms with consistent performance across operational conditions.

Therefore, this work aims to (i) determine the optimal hydrogel-to-feedstock ratio for maximizing methane production from FW, (ii) evaluate the resilience of hydrogel-based additives under increasing OLRs, and (iii) investigate how these formulations shape microbial community dynamics to drive performance gains. By upcycling agricultural waste into high-value bioenergy applications, this research introduces a novel additive platform that enhances AD performance while driving innovation in sustainable waste-to-energy technologies, offering a cost-effective, environmentally sound solution that supports the circular bioeconomy.

2. MATERIALS AND METHODS

2.1. Food Waste, Functional Materials, Anaerobic Sludge Characterization. The hydrogel production method follows the protocol established by our research collaborator.[26] The hydrogel-based cultivation substrates were initially developed to enhance choy sum plant yield by incorporating biochar and LECA into the growth matrix. Following the harvesting of choy sum plants, the waste hydrogel-based materials were collected and processed for reuse in AD applications. To ensure reproducibility and minimize batch-to-batch variability in hydrogel properties, all hydrogel materials used in this study were prepared following a standardized protocol under controlled conditions (e.g., fixed okara-to-cross-linker ratio, pH, and drying temperature). The physicochemical properties of the okara matrix used for hydrogel synthesis have been previously characterized in detail.[26] Triplicate AD reactors were also employed throughout the experiments to account for any residual variability and ensure statistical robustness of the AD performance results.

The hydrogels were dried at 105 °C to achieve a constant weight for total solids (TS) determination and then incinerated at 550 °C for 2 h to measure volatile solids (VS) content. Elemental composition analysis of the hydrogel-based materials was conducted using a

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Table 1. Characteristics of Sludge, Food Waste, and Waste Hydrogel Derived Materials[a]

Parameters	FW	Pure hydrogel (PH)	Biochar-hydrogel (BH)	LECA-hydrogel (LH)	Sludge
C (wt %)	51.98 ± 2.32	41.22 ± 2.37	43.69 ± 1.50	41.93 ± 2.87	32.85 ± 0.16
H (wt %)	7.62 ± 0.06	4.84 ± 0.06	2.23 ± 0.02	3.39 ± 0.20	4.75 ± 0.10
N (wt %)	4.04 ± 0.24	1.23 ± 0.01	1.18 ± 0.21	1.12 ± 0.09	5.43 ± 0.08
S (wt %)	0.15 ± 0.04	0	0.08 ± 0.01	0.09 ± 0.01	1.47 ± 0.05
O* (wt %)	36.21	52.71	52.82	53.47	55.5
TS (wt %)	25.54 ± 0.19	0.94 ± 0.01	15.24 ± 1.77	17.79 ± 1.36	5.85 ± 0.13
VS (wt %)	23.04 ± 0.23	0.90 ± 0.03	8.29 ± 0.59	11.28 ± 0.67	4.37 ± 0.14
VS/TS ratio	0.902	0.957	0.544	0.634	0.747
C/N ratio	12.87	33.51	37.03	37.44	6.05
pH	-	-	-	-	7.74 ± 0.05
TAN (g/L)	-	-	-	-	1.00 ± 0.12
SCOD (g/L)	-	-	-	-	1.71 ± 0.35

[a]All values in the table are presented as mean ± standard deviation (SD), based on triplicate measurements (n = 3).

FlashSmart CHNS Elemental Analyzer (ThermoFisher Scientific, USA) to quantify carbon (C), hydrogen (H), nitrogen (N), and sulfur (S) content (Table 1). PH contained 41.22 wt % C, 4.84 wt % H, and 1.23 wt % N, with a low VS content of 0.9 wt % and a C/N ratio of 33.51. BH exhibited slightly higher C content (43.69 wt %) but lower H (2.23 wt %) and N (1.18 wt %) compared to PH, along with a substantially higher VS content (8.29 wt %), attributable to the presence of biochar's inert C fraction. LH had a comparable C content to PH (41.93 wt %) and the higher VS content among the three (11.28 wt %), likely due to the combined contribution of organic matter and the porous structure of LECA, which may retain more biodegradable material. These compositional differences are relevant for interpreting their respective influences on AD performance.

The FW used in this study was collected from the Fine Food cafeteria at the National University of Singapore (NUS) and consisted primarily of postconsumers leftovers, including cooked rice, noodles, mixed vegetables, and meat or fish portions. Nonbiodegradable components such as plastic, bones, and packaging materials were manually removed before homogenizing the remaining waste and storing it at −20 °C to prevent microbial degradation prior to use. No additional chemical or thermal pretreatment was applied, as postconsumer food waste is inherently rich in readily biodegradable substrates. The anaerobic sludge inoculum was obtained from the mesophilic anaerobic digesters at the Ulu Pandan Water Reclamation Plant, Singapore. Before the AD experiments, FW and sludge were thawed at room temperature and characterized. TS content was determined by drying at 105 °C, and VS content was measured by incineration at 550 °C. The physicochemical properties of FW, sludge, and hydrogel-based materials, including their C/N ratio, pH, and soluble chemical oxygen demand (sCOD), are summarized in Table 1.

2.2. Semicontinuous AD Test. The semicontinuous AD test was conducted in 800 mL glass bottles with a working volume of 500 mL, consisting of 300 mL of inoculum and 200 mL of tap water. The hydrogel-based additives (PH, BH and LH) were preweighed to target concentrations of 20 and 40 wt % of total feed wright, then manually mixed with FW to ensure homogeneous distribution before loading to the reactors. The remaining feed proportion (60−80 wt %) consisted of FW, with exact ratio adjusted according to the additive concentration (Table 2).

The OLR was progressively increased in three stages: 0.3 g VS/L/d (Stage 1), 0.6 g VS/L/d (Stage 2), and 0.9 g VS/L/d (Stage 3). Each stage lasted 20 days to allow sufficient time for microbial communities to adapt to the increased substrate load and to ensure steady-state conditions before moving to the next stage. This stepwise increase was implemented to evaluate process resilience, identify any signs of overloading (e.g., VFAs accumulation or pH drop), and assess how methane yield and process stability respond to higher loading pressures under different additive treatments. The experiments were conducted in triplicate for each formulation and OLR stage, and results are presented as mean ± standard deviation (SD).

Table 2. Composition of Different Additive Formulations and OLR in the Semicontinuous AD Test

	PH (wt %)	BH (wt %)	LH (wt %)	FW (wt %)	OLR (g VS/L/d)
PH20	20	0	0	80	0.3, 0.6, and 0.9
PH40	40	0	0	60	0.3, 0.6, and 0.9
BH20	0	20	0	80	0.3, 0.6, and 0.9
BH40	0	40	0	60	0.3, 0.6, and 0.9
LH20	0	0	20	80	0.3, 0.6, and 0.9
LH40	0	0	40	60	0.3, 0.6, and 0.9

2.3. Chemical Analysis of Semicontinuous AD Test. To evaluate the performance and stability of the AD system, samples were collected every 2 days for the measurement of pH, sCOD, free organic acids/total inorganic carbon (FOS/TAC) ratio, ammonia nitrogen (NH_4^+-N), and VFAs content. Briefly, pH was monitored to assess acid−base balance, sCOD to determine the concentration of soluble organic matter available for microbial conversion, and the FOS/TAC ratio to evaluate system stability and buffering capacity. NH_4^+−N was measured to monitor nitrogen release and identify potential risks of ammonia inhibition, while VFAs were analyzed to track intermediate metabolites and detect possible accumulation that could hinder methanogenesis. Methane (CH_4) concentration in the biogas phase was determined using a gas chromatograph (Clarus 580 GC, PerkinElmer, USA) equipped with a thermal conductivity detector (TCD) and operated with argon and air as carrier gases. The sCOD in the liquid phase and the NH_4^+−N concentrations were determined using a HACH colorimeter (HACH DR900, USA) following the HACH standard method. The FOS/TAC ratio was calculated from total alkalinity and total VFAs content determined via titration using a TitroLine 5000 Automatic Titrator (HeGo Biotech, Germany). Individual VFA concentrations were quantified using a gas chromatograph (Clarus 580 Arnel, PerkinElmer, USA) equipped with a flame induction detector (FID).

2.4. Microbial Community Analysis. Samples for microbial community analysis were collected from the seed sludge and from each treatment group (PH20, PH40, BH20, BH40, LH20, and LH40) at the end of each OLR stage: day 20 (Stage 1, 0.3 g VS/L/d), day 40 (Stage 2, 0.6 g VS/L/d), and the final day of the experiment, day 60 (Stage 3, 0.9 g VS/L/d). These time points were selected to capture microbial community succession and adaptation in response to increasing substrate loading. Genomic DNA was extracted from each sample using the Soil DNA Isolation Plus Kit (Norgen Biotek Corp., Canada), following the manufacturer's protocol to ensure high yield and purity. The extracted DNA was stored at − 20 °C until analysis. The V3−V4 hypervariable regions of the 16S rRNA gene were amplified by polymerase chain reaction (PCR) to target both bacterial

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and archaeal populations associated with hydrolysis, acidogenesis, acetogenesis, and methanogenesis. The V3−V4 region was selected because it provides high phylogenetic resolution for both bacterial and archaeal taxa, enabling accurate identification of key functional groups involved in anaerobic digestion, including hydrolytic and fermentative bacteria, syntrophic acetogens, and methanogenic archaea. Amplicon sequencing was performed by NovogeneAIT Genomics (Singapore) using the Illumina NovaSeq 6000 platform, generating paired-end reads for downstream bioinformatics analysis.

3. RESULTS AND DISCUSSIONS

3.1. Function of PH, BH, and LH in Enhancing Methane Production under Varying OLRs. Optimizing methane production in AD relies on balancing substrate availability, microbial activity, and process stability. Functional materials like hydrogels, biochar, and LECA enhance AD efficiency by improving microbial interactions, buffering capacity, and VFAs conversion. However, their performance is strongly influenced by both additive concentration and OLR, as these factors govern microbial adaptation rates, syntrophic activity, and metabolic pathway dominance.

Herein, Figure 1 shows the impact of different additives on cumulative methane yield (CMY), where higher additive

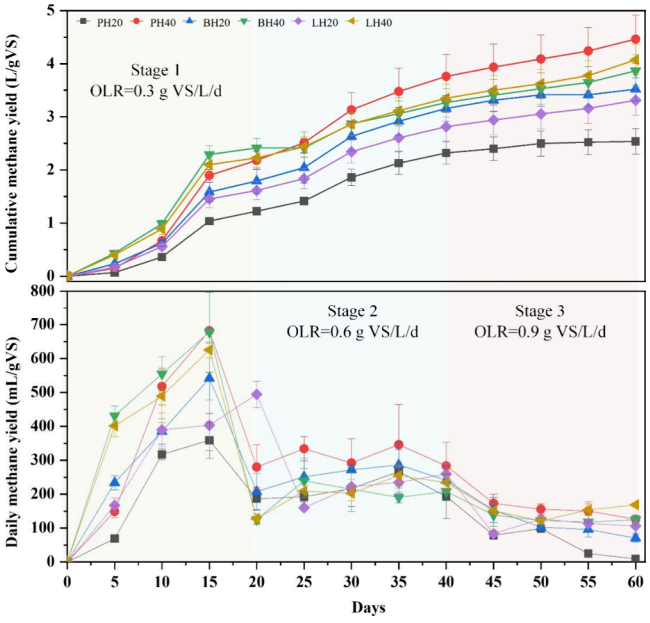


Figure 1. Cumulative and daily methane yields across different additive groups (PH, BH, LH) under varying OLRs.

concentration (40 wt %) consistently improved methane yield compared to 20 wt % for all additive types, indicating that greater additive availability enhances surface area for microbial colonization, buffering potential, and process stability. PH40 achieved the highest yield (4.07 ± 0.28 L/g VS), while PH20 had the lowest (2.54 ± 0.22 L/g VS), highlighting the synergistic influence of additive concentration and OLR on methane productivity. At higher OLRs, PH40 outperformed LH40 and BH40, whereas at lower OLRs, BH40 exhibited the highest CMY. At low OLRs, biochar-based additives (BH40) improved methane production, likely due to their capacity to enhance microbial attachment, DIET, and buffering capacity.[27] Conversely, at high OLRs, PH40 exhibited superior performance, indicating that hydrogel facilitated process stability by preventing excessive acidification and maintaining microbial homeostasis.[26] The intermediate performance of LH40 across OLRs suggests that LECA-hydrogel provided a balance between microbial attachment and mass transfer improvement.[28] This suggests that the dominant functional mechanism of each additive shifts with loading conditions (OLR), with biochar excelling under low substrate stress via DIET enhancement and buffering, while hydrogel sustains stability at higher loadings by mitigating acidification.

Under low OLR conditions (0.3 g VS/L/d), BH40 achieved the highest methane yield (3.71 ± 0.21 L/g VS), producing 27.9% more methane than PH40. This superior performance is likely linked to the biochar-rich hydrogel's dual functionality: (i) its high electrical conductivity, which facilitates DIET between fermentative bacteria and hydrogenotrophic methanogens (e.g., *Methanobacterium* spp.), enabling faster electron flow and reduced reliance on diffusible intermediates,[8] and (ii) its buffering capacity and porous architecture, which provide stable microhabitats for syntrophic partners.[23] While syntrophic acetate oxidation (SAO) is an important pathway in high-ammonia or thermophilic systems,[6] the low OLR and mesophilic conditions in this study, together with the enrichment of DIET-associated taxa (e.g., *Geobacter*, *Methanobacterium* spp.), suggest that DIET was the predominant mechanism. This is further supported by the observed methane yield enhancement without significant acetate accumulation, indicating efficient direct electron exchange rather than acetate oxidation as the primary route to methane formation. LH40, while slightly less effective than BH40, still outperformed PH40, suggesting that the LECA-hydrogel's porosity provided adequate microbial attachment sites and moderate buffering, resulting in stable but not peak performance. PH40 exhibited the lowest CMY in this phase, likely because hydrogel's slower microbial colonization rate and limited conductivity reduced early stage methanogenesis efficiency. These findings align with previous studies that demonstrated biochar's ability to enhance methane production by facilitating DIET between syntrophic bacteria and methanogens.[6]

At higher OLRs (0.6−0.9 g VS/L/d), PH40 outperformed BH40, with a ∼ 32.6% higher methane yield, and maintained superior performance throughout the experiment. This enhanced stability is attributable to the hydrogel's dual functional properties: (i) its high moisture-retention capacity, which maintains consistent reactor hydration and dilutes accumulated VFAs, and (ii) its intrinsic buffering ability, likely derived from proteinaceous and mineral components, which neutralizes excess acidity generated during rapid hydrolysis and acidogenesis.[29] Together, these mechanisms moderated pH fluctuations under elevated substrate loads, preventing process acidification, preserving methanogen viability, and supporting balanced syntrophic interactions, thereby enabling stable methane production at higher OLRs.[30] In contrast, BH40 performance declined under high OLR conditions, likely due to mass transfer limitations arising from its biochar-rich matrix. Higher OLRs caused VFAs and soluble organics to accumulate, creating diffusion barriers that limited effective substrate penetration into the biochar's internal pores.[31] This reduced accessibility diminishes the DIET advantage by limiting contact between fermentative bacteria and hydrogenotrophic methanogens, thereby lowering methane conversion efficiency. In comparison, PH40s hydrogel matrix exhibited high water-retention and swelling capacity, which enhanced bulk-phase contact between microbes and substrates,[32] minimizing diffusion resistance even under VFAs-rich conditions. Its

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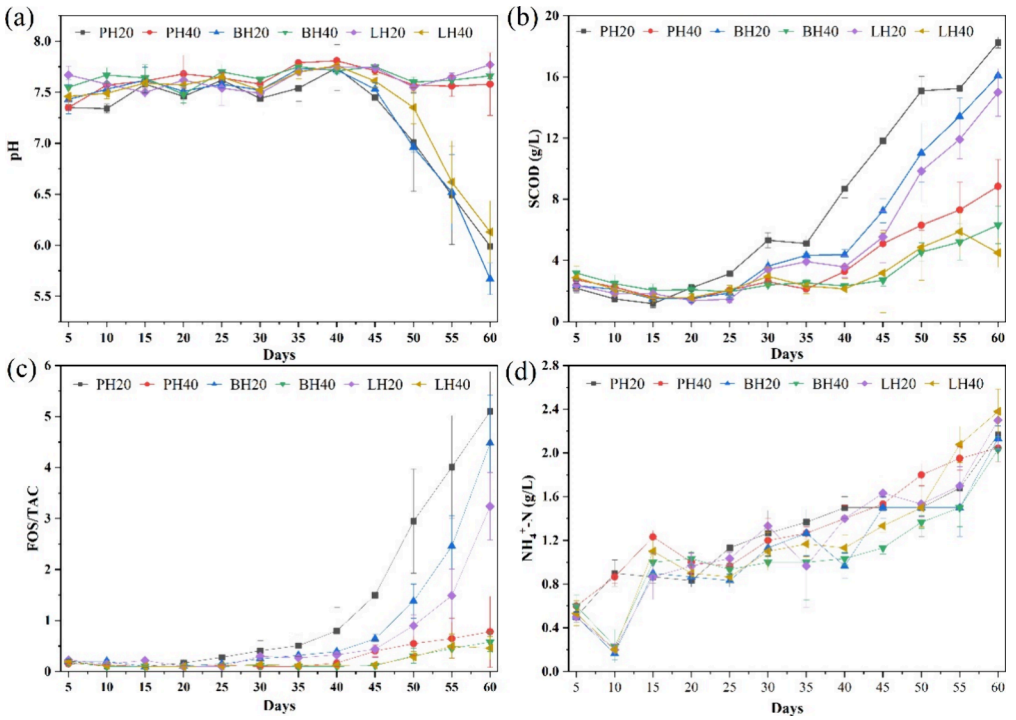


Figure 2. Performance metrics of the semicontinuous AD system under varying OLRs: (a) pH levels, (b) sCOD, (c) FOS/TAC ratio, and (d) NH_4^+−N concentrations.

protein-derived buffering components further mitigated localized acidification at the microbe−substrate interface, supporting sustained methanogenic activity.[33] LH40 exhibited moderate performance across all OLRs, indicating a balance between microbial attachment potential and buffering capacity. However, its lower conductivity compared to BH40 and less pronounced pH stabilization compared to PH40 may have limited its ability to offset diffusion constraints under high OLRs, resulting in steady but not peak methane production.

The daily methane yield (DMY) trends further insight into the stability and efficiency of the AD system across treatments. In the initial phase (Days 0−20), BH40 recorded the highest DMY, indicating rapid microbial colonization and effective DIET facilitation, consistent with biochar's role in promoting syntrophic interactions. However, PH40 displayed a steady upward trend in DMY, surpassing both BH40 and LH40 by Day 25, where its methane yield was 37.16% higher than LH20 at this point. This progressive increase highlights PH40s ability to adapt to rising OLRs and maintain methanogenic activity under substrate-rich conditions.

In the later stages (Days 20−55), PH40 sustained the highest and most stable DMY, demonstrating enhanced process resilience through moisture regulation, pH buffering, and mitigation of VFAs accumulation. In contrast, BH40s DMY declined at higher OLRs, likely due to substrate entrapment within biochar pores and reduced accessibility for microbial degradation.[34] LH40 maintained moderate and consistent DMY across all stages, reflecting a balanced contribution of microbial attachment and acidification control.

The microbial mechanisms underlying the differences in methane production between PH, BH, and LH can be attributed to their distinct effects on microbial attachment, DIET facilitation, and pH buffering. Biochar-based additives, particularly BH40, enhanced microbial colonization by providing a porous surface that facilitated the attachment of methanogenic archaea, such as *Methanosaeta* and *Methanobacterium*. This resulted in increased methane production under low OLRs, as previously reported by Bu et al.[8] However, at higher OLRs, hydrogel-based additives such as PH40 demonstrated superior performance due to their ability to maintain stable pH conditions, regulate moisture content, and prevent excessive VFA accumulation.

The results of this study suggest that tailoring additive selection to specific OLR levels can significantly enhance methane production efficiency in AD systems. For low OLR conditions (≤0.3 g VS/L/d), biochar (BH40) is the most suitable additive, as it promotes microbial attachment, enhances electron transfer, and provides buffering capacity against pH fluctuations. For moderate to high OLR conditions (0.6−0.8 g VS/L/d), hydrogel-based additives (PH40) are preferable due to their ability to regulate moisture retention, stabilize pH, and mitigate acidification risks. LECA-hydrogel (LH40) offers a balanced approach, making it a viable option for applications that require flexibility in OLR management.

3.2. Performance of the Semicontinuous AD System.

The performance and stability of the semicontinuous AD system were evaluated through four key operational parameters: pH stability, sCOD, FOS/TAC ratio, and NH_4^+-N levels, as illustrated in Figure 2. pH serves as a primary indicator of process balance, as it reflects the interplay between acidogenesis-driven VFAs production and buffering capacity.[35] Excessive VFAs accumulation without adequate alkalinity causes a sharp pH drop, inhibiting methanogenesis and potentially leading to process failure.[36] sCOD represents the concentration of readily biodegradable organic matter available for microbial metabolism, providing insights into substrate utilization efficiency and the extent of organic matter conversion.[37] The FOS/TAC ratio acts as a stability index, where values within the optimal range (typically 0.3−0.4) indicate balanced acid production and buffering capacity, while

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higher ratios signal acid accumulation and potential instability.[38] NH_4^+-N levels are monitored to assess nitrogen availability for microbial growth as well as to detect potential ammonia inhibition when concentrations exceed inhibitory thresholds.[39]

In Figure 2a, pH remained stable in all groups during Stages 1 and 2, ranging from 7.35 ± 0.06 in the PH40 group to 7.77 ± 0.08 in the LH20 group. However, as the OLRs increased to 0.9 g VS/L/d, the LH40, PH20, and BH20 groups exhibited a sharp decline in pH, indicating acidification and system imbalance. The BH40, PH40, and LH20 groups maintained relatively stable pH levels, demonstrating their superior buffering capacities. The improved stability in these groups could be attributed to the presence of biochar and hydrogel-based additives, which have been reported to facilitate microbial acclimatization and enhance system resilience against acidification.[30]

In Figure 2b, the PH20 group exhibited the highest sCOD levels throughout the experiment, with a peak of 3.14 g/L on Day 25, which continued to accumulate until the end of the study. By Day 60, the sCOD content in PH20 was 2.89 times higher than in PH40, confirming that the additive dosage significantly influences the balance of the AD system. A similar trend has been reported in previous studies, where higher sCOD levels were linked to insufficient methanogenic activity and VFAs accumulation, leading to lower methane yields.[40]

The FOS/TAC ratio serves as an early warning indicator for AD system stability. On Day 60, the PH20 and BH20 groups recorded FOS/TAC values (Figure 2c) of 5.1 ± 0.78 and 4.48 ± 0.94, respectively, suggesting that microbial communities in these reactors were unable to neutralize the accumulating acids. Conversely, PH40, BH40, and LH20 maintained FOS/TAC values below 2.5, indicating a well-buffered system capable of sustaining methanogenesis. The buffering ability of PH40 and BH40 may be linked to their enhanced electron transfer properties and improved microbial syntropy, which have been shown to mitigate acid accumulation and stabilize AD performance.[41]

In our study, NH_4^+-N concentrations (Figure 2d) increased progressively across all groups, reflecting the continuous degradation of protein-rich food waste and subsequent release of amino acids. By the end of the experiment, NH_4^+-N levels ranged from 2.03 ± 0.11 g/L in BH40 to 2.38 ± 0.20 g/L in LH40, with LH40 experiencing a 110.62% increase in the final stage. Excessive NH_4^+-N concentrations can inhibit methanogenesis by disrupting microbial cell function and altering enzymatic activity.[39] However, PH40 and BH40, which demonstrated higher methane yields, exhibited relatively lower NH_4^+-N levels, suggesting that their microbial consortia effectively utilized ammonia for growth and metabolic activity. This aligns with previous findings that biochar and hydrogel additives can enhance ammonia assimilation, reducing its inhibitory effects on methanogens.[42]

Overall, these results highlight the critical role of additive dosage in maintaining system stability under varying OLRs. While lower additive concentrations (20%) were insufficient to buffer against acidification, higher concentrations (40%) contributed to a more stable AD process.

3.3. VFAs Consumption in Different Groups with Changed OLR. VFAs are critical intermediates in AD, influencing both process stability and methane production efficiency.[34] Figure 3 presents the VFAs concentrations across different groups under varying OLRs. Herein, in Stage 3, when

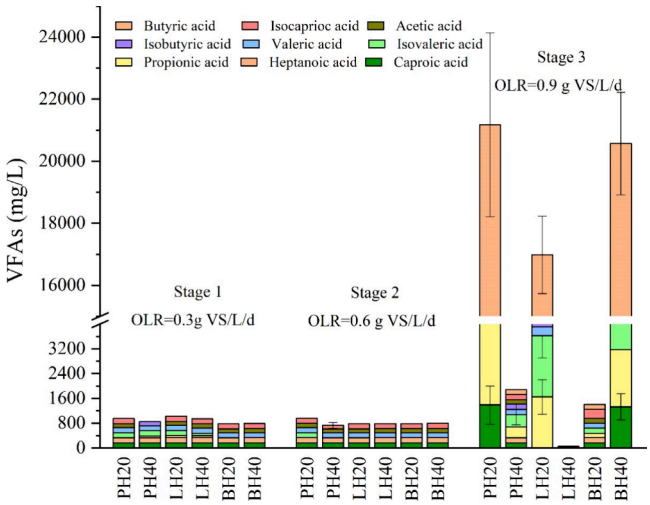


Figure 3. VFAs concentrations across different groups under varying OLRs.

the OLRs increased to 0.9 g VS/L/d, VFAs accumulation was significantly elevated in most groups, indicating that elevated OLR led to organic overloading and a reduced capacity of methanogens to convert acids into methane. The PH20 group exhibited the highest VFA concentration at 21,170.02 mg/L, dominated by acetic acid, suggesting severe inhibition of methanogenic activity and poor process stability. Similar findings have been reported by Lim et al.[34] where high OLRs resulted in VFA build-up due to the imbalance between acidogenic and methanogenic bacteria. The phenomenon of inhibition at elevated OLRs is primarily attributed to the accumulation of acidic intermediates, which lower the pH and negatively affect methanogenic communities.[37]

Distinct VFA profiles were observed among additives under high OLR, indicating different metabolic responses and degradation pathways. In the PH20 group, acetic acid (130.11 mg/L) predominated, with propionic acid absent, implying disruption of syntrophic acetate oxidation. The PH40 group, in contrast, showed a higher proportion of isobutyric acid, suggesting a microbial shift toward branched-chain fatty acid production, possibly due to altered community structure under additive influence.[43] The LH20 also displayed elevated acetic acid levels (127.28 mg/L), indicating a pathway bias toward acetogenesis, whereas LH40 exhibited a more diverse profile, with both acetic acid and isovaleric acid as major VFAs, a pattern associated with active amino acid degradation and mixed-substrate utilization.[44] In the BH20 group, valeric acid was dominant and propionic acid absent, pointing to selective inhibition of propionate-degrading bacteria under stress conditions. BH40 exhibited a similar trend, dominated by acetic acid, indicating sustained acetoclastic methanogenesis despite high OLR, likely supported by biochar's surface area for microbial colonization and pH buffering capacity. These variations in VFAs profiles highlight the distinct metabolic responses induced by different additives, which influence the rate and extent of VFAs consumption in FW-AD systems.

Notably, PH40 and LH40 demonstrated superior performance in VFA consumption during Stage 3, suggesting that specific additives could mitigate the inhibitory effects of high OLR by facilitating more efficient VFAs uptake and conversion. Xu et al.[22] reported that biochar and chitin materials could enhance methane yield by promoting VFA

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Table 3. Alpha Diversity Indices of Microbial Communities in Different Treatment Groups under Varying OLRs, Compared to the Initial Inoculum

Sample name	Chao1	Dominance	Goods coverage	Observed features	Pieloue	Shannon
Seed sludge	1141.28	0.08	1.00	1017.00	0.56	5.54
PH40-Stage 1	550.06	0.10	1.00	541.00	0.56	5.09
PH40-Stage 2	674.67	0.11	1.00	610.00	0.54	4.97
PH40-Stage 3	453.78	0.12	1.00	452.00	0.57	5.00
BH40-Stage 1	1056.95	0.07	1.00	968.00	0.57	5.63
BH40-Stage 2	490.40	0.11	1.00	483.00	0.52	4.62
BH40-Stage 3	662.07	0.05	1.00	618.00	0.60	5.60
LH40-Stage 1	824.93	0.08	1.00	722.00	0.54	5.13
LH40-Stage 2	751.46	0.11	1.00	635.00	0.52	4.80
LH40-Stage 3	1006.70	0.15	1.00	954.00	0.55	5.40

degradation and stabilizing the microbial community. Biochar, in particular, has been recognized for its ability to adsorb VFAs, buffer pH fluctuations, and provide a conducive surface for microbial attachment, thereby improving syntrophic interactions between acidogenesis and methanogens.[36] These results further reinforce the potential of biochar and other additives in enhancing the resilience of AD systems under fluctuating OLR conditions.

Overall, PH40 and LH40 demonstrated the most effective VFAs consumption under increased OLR, as they maintained lower residual VFAs concentrations compared to other groups, suggesting improved microbial adaptation and methanogenic performance. BH20 also showed relatively stable VFAs consumption, particularly in the degradation of valeric acid, while BH40 exhibited efficient acetic acid utilization, indicating enhanced acetoclastic methanogenesis. These findings highlight the importance of selecting suitable additives such as biochar to mitigate VFAs accumulation and ensure stable methane production under varying OLR conditions.

3.4. Microbial Community Analysis. *3.4.1. Alpha Diversity Indices and Principal Component Analysis (PCA).* Alpha diversity metrics offer valuable insights into the microbial community structure within AD systems, which are closely linked to process performance and methane production. In this study, the Chao1 index, estimating species richness, decreased from 1141.28 in the initial inoculum to 490.40 in the BH40 group during stage 2, indicating a substantial reduction in microbial richness post-AD. The dominance index, which reflects species evenness, was lowest (0.08) in both the initial inoculum and LH40 during Stage 1, suggesting a relatively balanced community distribution at these points. The Shannon index, representing overall diversity, ranged from 4.62 in BH40 (Stage 2) to 5.63 in BH40 (Stage 1), highlighting that operational conditions and additive type influenced community complexity (Table 3).

The observed decline in microbial diversity following AD suggests a selection process favoring specific hydrolytic and methanogenic microorganisms essential for efficient biogas production. This aligns with findings that specialized microbial communities with high numbers of species-level operational taxonomic units (sOTUs) are crucial for achieving high methane yields.[45]

Principal Component Analysis (PCA) further revealed clear separation between the initial inoculum and all post-AD samples (Figure 4), reflecting the transition from a broad, diverse seed community to more functionally streamlined communities. Notably, samples from the PH groups clustered closely with other treatment groups in the PCA plot,

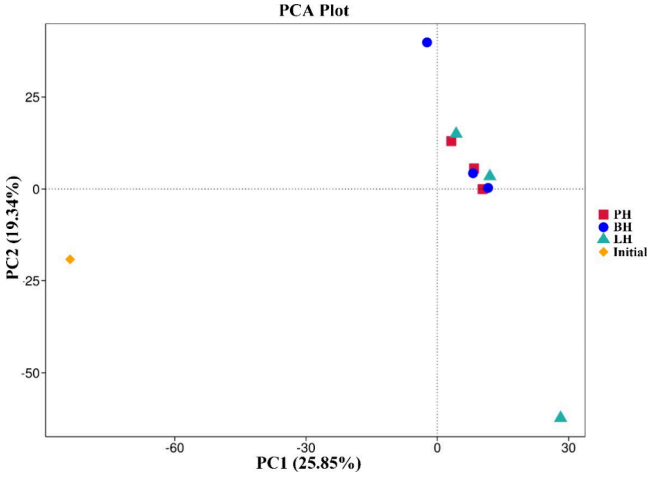


Figure 4. PCA illustrating the microbial community composition across different treatment groups and the initial inoculum.

suggesting a convergent microbial structure likely influenced by hydrogel supplementation. This effect may be attributed to the chitosan content within the hydrogel, which can enhance methanogen aggregation, improve syntrophic partner proximity, and facilitate DIET. Variations in clustering within the PCA plot also suggest that substrate composition and additive-specific properties shaped community assembly, with biochar- and LECA-based groups exhibiting slightly more dispersed clustering, possibly reflecting niche differentiation in microbial colonization and metabolic roles. These findings are consistent with studies indicating that microbial community shifts in AD are strongly influenced by feedstock type and operational conditions.[46]

Overall, these alpha diversity and PCA results demonstrate that additive type and OLR conditions influenced microbial community structure, with hydrogel-based additives steering the community toward a more cohesive and syntrophically efficient configuration that supports enhanced methanogenesis.

3.4.2. Relative Abundance of Anaerobic Bacteria. As shown in Figure 5a–b, the microbial community composition underwent significant restructuring following AD of FW, with clear shifts observed both across OLR stages (Figure 5a) and among hydrogel-based additive groups (Figure 5b) compared to the initial inoculum.

OLR was a major driver of microbial dynamics in the semicontinuous AD system. Across all groups, the highest relative abundance of *Bacteroidia* and *Gammaproteobacteria* was observed at OLR = 0.6 g VS/L/d (Stage 2), coinciding

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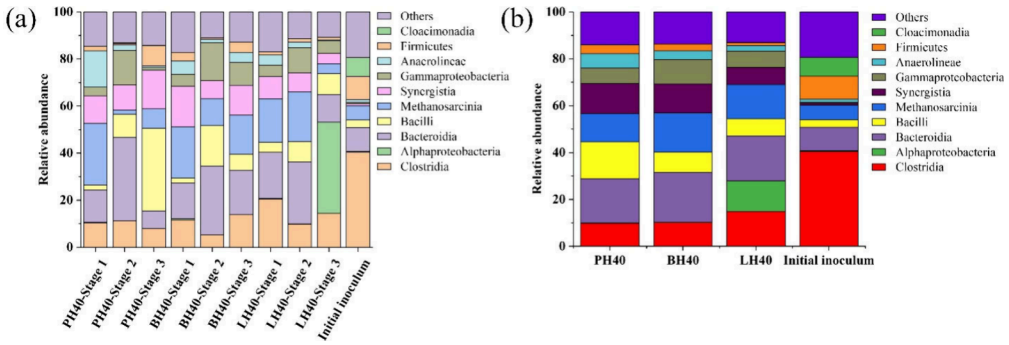


Figure 5. Relative abundance of bacterial communities at the phylum level in different groups: (a) variation across OLRs and (b) comparison among different additive materials.

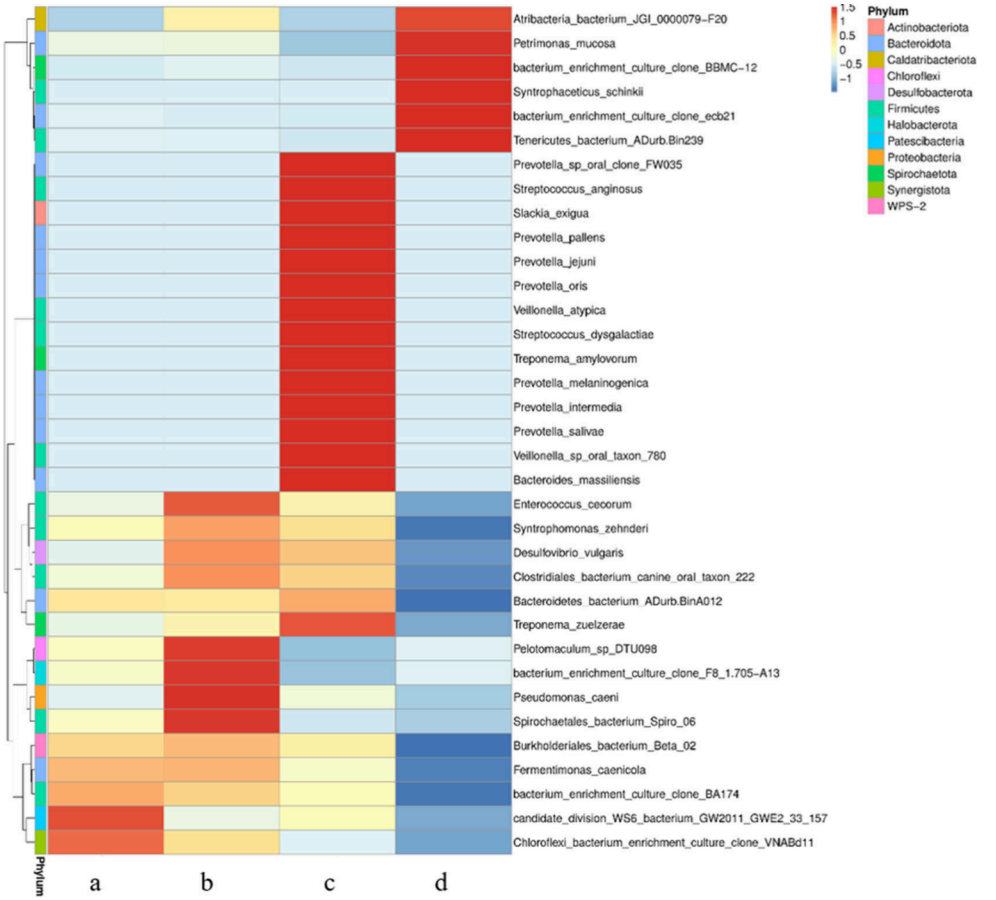


Figure 6. Relative abundance of the top 35 bacterial species across different treatment groups: (a) PH, (b) BH, (c) LH, and (d) initial inoculum.

with peak VFAs conversion and methane production rates. In contrast, *Bacilli* abundance was lowest at OLR = 0.3 g VS/L/d (Stage 1), likely due to limited substrate availability suppressing fermentative activity and downstream methanogenesis. These trends indicate that moderate OLR levels favor substrate-degrading microbial groups, while extreme values can cause community imbalance and suboptimal performance.[47]

Beyond OLR effects, the type of hydrogel-based additive markedly influenced microbial composition. The microbial community underwent substantial restructuring following AD of FW in all additive groups (PH40, BH40, and LH40), as compared to the initial inoculum. A substantial decrease in the relative abundance of *Clostridia* was observed, by 75.66%, 74.71%, and 63.05%, respectively (Figure 5a), indicating a typical microbial succession process within AD systems. *Clostridia* are known fermentative bacteria involved in hydrolysis and acidogenesis.[37] Their decline reflects the transition from early stage degradation to later stages favoring methanogenic populations.[48]

Enrichment was observed in *Bacteroidia*, *Bacilli*, *Methanosarcina*, and *Anaerolineae* across all treatment groups. *Bacteroidia* and *Bacilli* are key players in the degradation of polysaccharides and proteins, supporting efficient substrate breakdown and VFAs formation.[2] The presence of *Methanosarcina*, a versatile methanogen capable of both acetoclastic and hydrogenotrophic pathways, is strongly correlated with improved methane yield under fluctuating OLR conditions.[49] The increased abundance of *Anaerolineae*, a member of the

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phylum *Chloroflexi*, is particularly significant, as it has been associated with syntrophic acetate oxidation and interspecies hydrogen transfer; both crucial for stable methanogenesis in high-strength waste systems.[50]

Among all groups, PH40, which achieved the highest methane yield, had the greatest enrichment of *Bacilli* (∼16%), followed by *Synergistia* (∼13%), *Firmicutes* (∼4%), *Methanosarcina* (∼10%) and *Anaerolineae* (∼12%). The co-occurrence of *Bacilli* and *Synergistia* is notable, as both are key fermentative taxa involved in the breakdown of complex organics and amino acids into acetate, hydrogen, and amino acid-derived intermediates, which feed downstream methanogenesis. *Bacilli* are facultative anaerobes that contribute not only to early stage hydrolysis of FW but also to pH buffering through metabolic versatility, consuming oxygen traces and producing alkaline metabolites that mitigate acidification under high OLR conditions.[51] *Synergistia* are frequently associated with syntrophic amino acid degradation and are known to establish obligate partnerships with hydrogenotrophic methanogens.[52] *Methanosarcina*, a metabolically versatile methanogen, is capable of both acetoclastic and hydrogenotrophic pathways, allowing it to thrive under fluctuating VFAs and hydrogen concentrations.[40] *Anaerolineae*, members of the phylum *Chloroflexi*, have been linked to syntrophic acetate oxidation and interspecies hydrogen transfer, supporting methanogenic stability in high-load AD systems.[53] The combined presence of these functional groups likely promoted efficient hydrolysis, acidogenesis, and syntrophic cooperation, contributing to PH40s superior methane yield under high OLR conditions.

BH40 displayed high *Bacteroidia* (∼29%) and *Methanosarcina* (∼13%) abundance, indicating strong hydrolytic and methanogenic potential. However, its methane yield was slightly lower than PH40, possibly due to reduced substrate accessibility within biochar pores, which could limit fermentative−methanogenic interactions. LH40 was dominated by *Bacteroidia* (∼35%) and *Anaerolineae* (∼16%), supporting moderate methane production via balanced substrate degradation and syntrophic acetate oxidation, indicating a functionally balanced but less methanogen-enriched community compared to PH40. These microbial community patterns align with the observed OLR-dependent performance trends, where the conductive and buffering properties of BH40 better sustained syntrophic activity and methane generation under low-load conditions, while the pH-stabilizing and syntrophic-enriching effects of PH40 promoted superior methanogenesis at higher substrate loads.

The porous, hydrated structure of the hydrogel-based additives likely provided a high surface area for microbial colonization, enabling spatially distinct microenvironments that supported both fermentative bacteria and methanogenic archaea. This physical−chemical property facilitated syntrophic interactions and stabilized microbial networks, even under varying OLR conditions. Additive-specific enrichment patterns, such as the *Bacilli*−*Synergistia*−*Methanosarcina* consortium in PH40 or the *Bacteroidia*−*Anaerolineae* partnership in LH40, highlight that the hydrogel matrix not only acted as a structural support but also modulated community assembly, promoting complementary metabolic functions that underpin stable and enhanced methane production.

At the species level, Figure 6 reveals a clear divergence in microbial community composition across the different treatments. The initial inoculum was dominated by *Artibacteria bacterium*, *Petrimonas mucosa*, *Syntrophaceticus schinkii*, and *Tenericutes bacterium*, which are typically associated with early stage fermentation processes. Following AD, the PH40 group was enriched in *Chloroflexi* bacterium, BH40 in *Pseudomonas caeni*, and LH40 in *Bacteroides massiliensis*, indicating substrate- and additive-specific microbial enrichment. These taxa are known to contribute to substrate degradation and syntrophic metabolism, reinforcing the role of hydrogel-based additives in shaping functional microbial niches.[9]

In summary, these results highlight the critical influence of both OLR and additive type on microbial community structure and function in semicontinuous AD systems. The enhanced abundance of methane-producing and syntrophic bacteria in PH40 correlates with its superior biogas yield, underscoring the potential of waste hydrogel materials to steer microbial consortia toward optimal methanogenesis. This microbial shift aligns with previous studies demonstrating the pivotal role of community structure in determining AD performance and resilience.[22] Collectively, these findings provide mechanistic insights into how low-cost functional additives derived from agricultural waste can be leveraged to enhance biogas production while promoting circular economy goals.

4. CONCLUSIONS

This study highlights the effectiveness of waste-derived hydrogel materials in enhancing AD of FW under varying OLRs. At low OLR (0.3 g VS/L/d), BH40 achieved the highest methane yield (3.71 ± 0.21 L/g VS), producing 27.9% more methane than PH40, primarily due to its biochar-rich matrix that provided enhanced buffering capacity and a conductive surface, fostering syntrophic partnerships between fermentative bacteria and methanogens even under limited substrate availability. At high OLR (0.9 g VS/L/d), PH40 outperformed all groups with the highest yield (4.07 ± 0.28 L/g VS), attributed to its pH-stabilizing properties, rapid VFAs turnover, and enrichment of methanogenic and syntrophic taxa such as *Bacilli*, *Synergistia*, and *Cloacimonadia*, which supported efficient hydrolysis, acidogenesis, and downstream methanogenesis under higher substrate loads.

These results demonstrate that the selection of functional additives must be tailored to OLR conditions to optimize biogas production and maintain microbial balance. Conductive, buffering biochar-based hydrogels (BH40) are better suited for low-load systems, where they enhance methanogenic stability by facilitating direct interspecies electron transfer and buffering pH. In contrast, pH-stabilizing, syntrophic-enriching protein-based hydrogels (PH40) excel under higher substrate loads, where they accelerate VFAs turnover, prevent acid accumulation, and enrich functional methanogenic consortia. Such OLR-specific additive selection ensures stable microbial community dynamics and sustained high methane yields.

Repurposing agricultural hydrogel waste as AD additives aligns strongly with circular economy principles by transforming a disposal-bound byproduct into a value-added functional material that enhances methane yield. This practice supports waste minimization, resource recovery, and renewable energy generation, while reducing reliance on virgin materials. Integrating agricultural residues back into productive use within AD systems reinforces a closed-loop model, where waste is continuously cycled into valuable outputs. Additionally, diverting agricultural waste from landfill or incineration contributes to greenhouse gas mitigation and advances sustainable waste management objectives.

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From a scalability perspective, both BH40 and PH40 have potential for integration into pilot- and full-scale digesters, as their production relies on abundant agricultural residues and relatively simple hydrogel preparation processes. The robust performance of these materials under varied OLRs suggests adaptability to different digester operational regimes, although long-term stability, reusability, and cost−benefit analyses should be assessed in larger-scale continuous systems. Future work shall focus on pilot-scale trials to validate performance under industrial conditions, life cycle assessments to quantify environmental benefits, and techno-economic analyses to assess cost-effectiveness. Additionally, exploring formulation optimization, regeneration potential, and compatibility with codigestion feedstocks will be key to ensuring sustainable and economically viable implementation.

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Notes

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