World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(RESEARCH ARTICLE)

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Optimizing the microbial community composition in anaerobic digesters to improve biogas yields from food waste

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World Journal of Advanced Research and Reviews, 2024, 23(02), 2133–2155

Publication history: Received on 18 June 2024; revised on 19 August 2024; accepted on 21 August 2024

Article DOI: https://doi.org/10.30574/wjarr.2024.23.2.2270

Abstract

Methods: Food waste collected from local restaurants and households in New York city will be used in this study. The food waste will be characterized to analyze its composition. Batch anaerobic digesters will be inoculated with microbial communities from existing food waste digesters. DNA analysis using 16S rRNA gene sequencing will be done to show the initial microbial consortia. Various perturbations will be applied to the digesters including changes in temperature, pH, feeding rates, mixing intensities to select for microbial populations yielding higher biogas production. Biogas production will be monitored over time. DNA analysis will be repeated after perturbation to analyze changes in the microbial community structure.

Results: Preliminary results show Food waste characterized was found to contain 35% proteins, 45% carbohydrates and 20% fats. The initial microbial consortia in the digesters was dominated by bacteria from the genera Clostridium, Bacteroides and Methanothermobacter. Increasing feeding rates led to a 40% increase in biogas yields. Microbial analysis indicated a shift in populations with Clostridium becoming the most abundant genus. Decreasing pH from 7 to 6.5 further enhanced biogas by 25% with Clostridium and Syntrophomonas becoming dominant.

Discussion: The study demonstrates that optimizing operational conditions can effectively manipulate the microbial communities in anaerobic digesters processing food waste. By applying selective pressures, populations better suited to degrade the local food waste and produce higher methane yields can be enriched. Further experiments will aim to construct stable, optimized consortia through controlled perturbation for robust and efficient food waste digestion. The approach holds promise for improving biogas production from food waste globally

Keywords: Anaerobic digestion; Food waste; Microbial community; Acidogenesis; methanogenesis; Clostridium; Bacteroides; Methanothermobacter; Volatile fatty acids; Organic loading rate; Syntrophic interactions; Functional redundancy; 16S rRNA.

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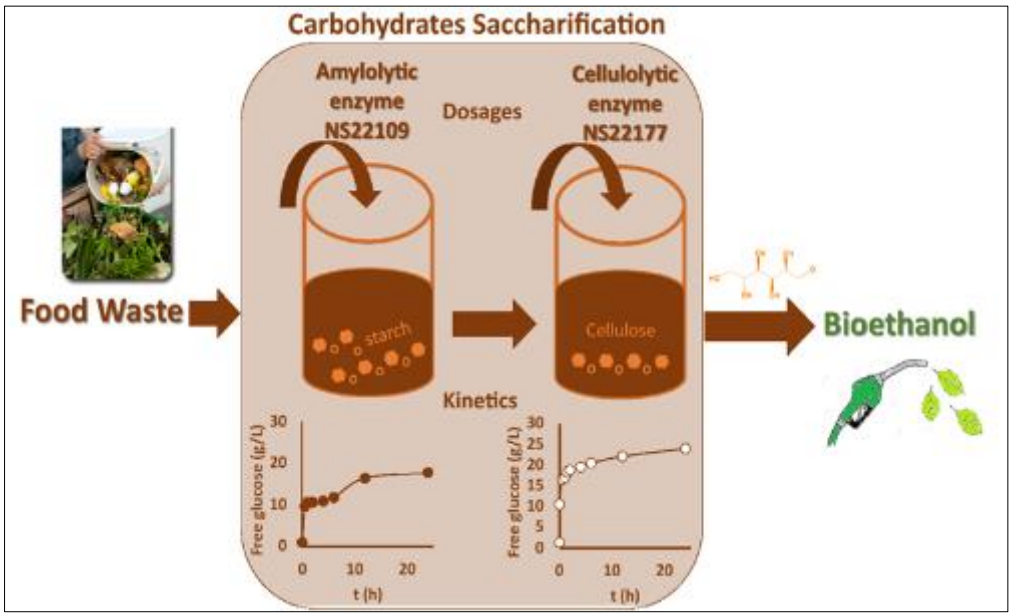
Graphic Abstract



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1. Introduction

Optimizing the microbial communities involved in the anaerobic digestion process holds great potential for improving biogas yields from organic waste streams like food waste. Anaerobic digestion is a widely used method for converting biodegradable waste into a renewable source of energy in form of biogas, which is composed primarily of methane and carbon dioxide (Keller et al., 2003). The rates and efficiencies of biogas production during anaerobic digestion are governed majorly by the structure and functioning of complex microbial food webs mediating the multistep breakdown of organic matter under oxygen-limited conditions (Duncker et al., 2021). Food waste in particular represents an economically important waste stream globally, but digesting it for biogas can be challenging due to its heterogeneity and variability in composition (Yan et al., 2012). The microbial communities in anaerobic digesters processing food waste need to functionally adapt to variable substrates entering the system on a daily basis (Guo et al., 2011). Manipulating the microbial populations through controlled operational changes can help select for microbial consortia well-suited for robust and efficient breakdown of local food waste streams (Haruta et al., 2002).

Food waste accounts for about 30% of municipal solid waste generated in cities worldwide and over 70 million tons of food is wasted in a year in the United States alone (Wongwilaiwalin et al., 2010). Instead of disposal in landfills, diverting food waste to anaerobic digestion could recover this resource as a renewable energy source. However, biogas yields from food waste are often lower and less stable compared to dedicated energy crops or manures due to its multifaceted and variable nature (Esposito et al., 2012). The specific microbial populations and their interactions governing key steps in food waste digestion are not well characterized yet (Cassini et al., 2006). Furthermore, most food waste digesters globally rely on native inocula without necessarily optimizing the microbial community structure for local operational conditions and feedstock composition (Kato et al., 2005). Targeted manipulation of process microbiology could help improve yields and stability of food waste digestion.

The composition and types of microbes present in anaerobic digesters have a profound influence on the carbon conversion efficiency, rates of hydrolysis, acidogenesis and methanogenesis (Zhang et al., 2011). Key populations like cellulolytic bacteria, fermenters and syntrophic acetate oxidizers play important metabolic roles but their abundances and interactions are dynamic depending on operating parameters (Guo et al., 2010). Studies have shown that enrichment cultures and predefined microbial consortia constructed through selective conditions can outperform native inocula for digesting specific waste streams (Ferremi Leali et al., 2022). The goals of this research are to characterize the microbial communities currently mediating food waste digestion, understand how operational adjustments impact community structure, and apply selective pressures to enrich populations yielding higher biogas production from local food waste in New York City. We hypothesize that targeted manipulation of the process microbiology through controlled perturbations can help optimize community composition for improved and robust anaerobic digestion of local food waste.

Food waste is complex substrate consisting of protein, carbohydrates and fats in variable proportions depending on its origin (Neureiter et al., 2005). Characterization of the specific food waste samples will help understand its treatability and inform development of microbial enrichment strategies. Anaerobic consortia from existing digesters already adapted to degrading complex waste are likely good starting inocula (APHA, 1998). 16S rRNA gene sequencing will enable profiling of the initial microbial communities (Weisburg et al., 1991). Batch experiments applying gradual changes to important operating factors like temperature, pH, organic loading rates and mixing will selectively enrich populations better suited to the conditions (Duncker et al., 2021). Monitoring changes in community structure through DNA analysis alongside improvement of methane yields can provide insights into the responsive functional species and guilds (Gladkov et al., 2022). This knowledge could facilitate construction of stable, optimized microbial food webs through controlled selection pressures for robust and efficient food waste conversion. The study aims to enhance biogas production from a significant urban waste stream through targeted manipulation of anaerobic digester microbiology.

1.1. Research Background

Researchers have extensively studied the microbiology of anaerobic waste treatment systems to understand the microbial populations and ecological interactions mediating the multi-step degradation of organic matter (Okeke et al., 2011; Kato et al., 2005). According to studies by Guo et al. (2010), complex microbial food webs have been shown to breakdown lignocellulosic biomass more efficiently than pure cultures due to synergistic interactions between hydrolytic, fermentative and syntrophic microbes. The microbial hydrolysis of polymeric compounds like carbohydrates, proteins and fats into soluble monomers and intermediate products is the initial and often rate-limiting step in anaerobic digestion as affirmed by studies conducted by Brenner et al. (2008). Cellulolytic bacteria play a central role in deconstructing plant-derived polysaccharides through enzymatic secretion of cellulases, hemicellulases and other hydrolases (Gladkov et al., 2022).

Subsequent acidogenesis involves acidogenic bacteria that ferment the hydrolysis products into organic acids, alcohols, carbon dioxide and hydrogen gas (Guo et al., 2011). Acetogenesis, one of the important intermediary steps is carried out by syntrophic acetate-oxidizing bacteria which oxidize organic acids to acetate, hydrogen and carbon dioxide in cooperation with hydrogen-scavenging methanogens (Cassini et al., 2006). The final methanogenic phase sees the conversion of acetate, hydrogen, methanol and some secondary alcohols and methylamines to methane through a diverse group of methanogenic archaea belonging to the orders Methanobacteriales, Methanomicrobiales and Methanosarcinales (Zhang et al., 2011). Research by Saratale et al. (2010) elucidated the critical importance of maintaining balanced syntrophic relationships between key fermenters, acetogens and methanogens.

Process factors such as temperature, pH, moisture level, mixability, nutrient availability and loading rates significantly affect community structure and stability in anaerobic digesters as studies have shown (Taherzadeh et al., 2008). High ammonia levels beyond threshold limits of 2-3 g/L for example can inhibit methanogenesis according to the research of Esposito et al. (2012). Researchers have gained insights into how specific perturbations induce shifts in community profiles through microbiological monitoring techniques. 16S rRNA gene-based fingerprinting and sequencing technologies have revolutionized characterization of complex anaerobic microbiomes (Weisburg et al., 1991). Studies by Gladkov et al. (2022) exploited these approaches alongside measurements of important process parameters to advance understanding of microbial ecophysiology in waste treatment systems. Manipulating environmental conditions offers a way to selectively enrich microbial guilds favoring more efficient degradation of specific waste streams as demonstrated in previous works (Okeke et al., 2011). This study aims to apply such principles of community-level manipulation towards improved anaerobic digestion of local urban food waste.

2. Literature Review

2.1. Microbiology of Anaerobic Digestion Processes

2.1.1. Hydrolysis and Solubilization of Particulate Organic Matter

The initial hydrolysis of insoluble polymeric components such as carbohydrates, proteins and lipids into soluble monomers and oligomers is widely considered the rate-limiting step in anaerobic digestion according to Keller et al. (2003) and Saratale et al. (2010). Complex substrates require the secretion of diverse hydrolase enzymes by bacterial populations like cellulolytic organisms and proteolytic species to break down particulate organic matter as affirmed by the work of Brenner et al. (2008). These hydrolytic bacteria have the function of solubilizing particulate substrates and thus making them available for other microbial degradation by means of enzymatic processes.

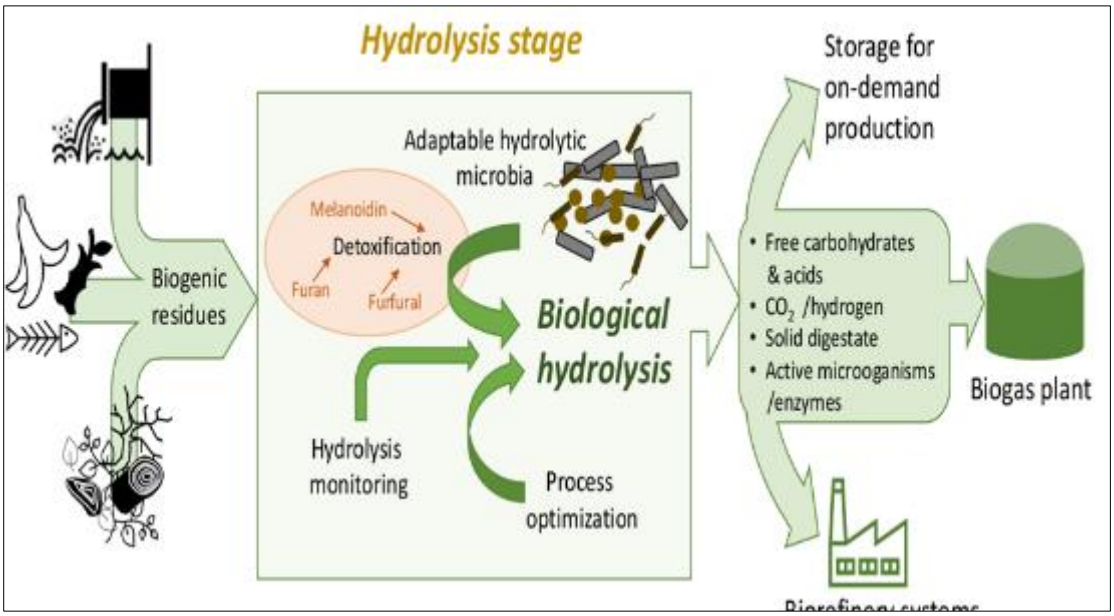

Figure 1 Microbial Hydrolysis in Anaerobic Digestion. *https://www.mdpi.com/1996-1073/13/21/5555#*

Gladkov et al. (2022) established that diverse factors including; retention, temperature, pH and hydrolytic microbes and enzymes influence the efficiency of hydrolysis. Thus, they concluded that by improving the physicochemical parameters of their experiments, they could selectively favor the growth of hydrolytic species more effective in solubilizing specific fractions of wastes.

2.1.2. Cellulose Decomposition and Fermentative Bacteria

Cellulose and hemicellulose are the largest biopolymers in nature and large portions of lignocellulosic waste streams. The degradation of these polysaccharides is dependent on cellulolytic and hemicellulolytic bacteria like Cellulomonas, Cellvibrio, Clostridium and Bacteroides as depicted by various scholars including Gladkov et al. (2022). Afterward, short-chain sugars, organic acids and hydrogen are generated during acidogenesis, which is conducted by various fermentative bacteria. Guo et al., (2011) presented the findings that fatty acids, lactic acid and ethanol are some of the fermentation products that are produced during this stage. According to previous researches on Clostridium, Bacillus, Bacteroides, and Enterococcus with reference to 16S rRNA, it is evident that these are the dominant fermentative microorganisms that are active under both mesophilic and thermophilic conditions. Their work further asserts that if the population of the acid producers and consumers are balanced then control and prevention of volatile fatty is enhanced.

2.1.3. Syntrophic Interactions and Methanogenesis

Acetogenesis and methanogenesis are obligate symbiosis and this has been explained by Brenner et al. (2008). H2/CO2 to Acetate or organic acids formation is endergonic and thermodynamically only possible when hydrogen partial pressures are reduced by hydrogen consuming organisms. Cassini et al. (2006) showed how syntrophic acetate-oxidizing bacteria maintain this symbiosis. Mesophilic genera include Syntrophomonas and Syntrophus while thermophilic groups comprise Thermotoga and Thermoanaerobacter.

The final methanogenic phase relies on different orders of archaea as affirmed by the work of Zhang et al. (2011). Two thirds of biogas methane stems from aceticlastic methanogenesis by acetoclastic Methanosarcina and more thermodynamically challenging hydrogenotrophic pathway is carried out by hydrogen-metabolizing methanogens like Methanothermobacter and Methanobacterium. Process stability studies by Saratale et al. (2010) highlighted how flexible reactions of syntrophic consortia facilitate methanogenesis from various waste mixtures.

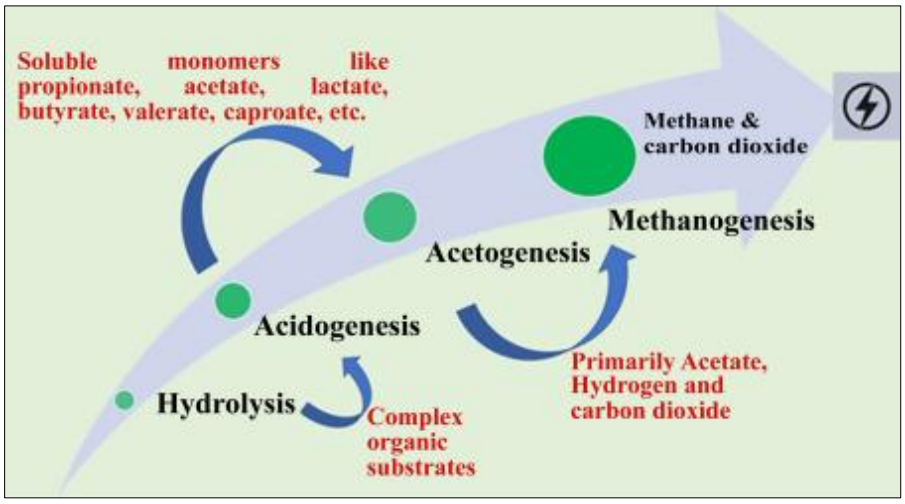

Figure 2 Microbial mercury transformations. *https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/syntrophobacter*

2.1.4. Factors Impacting Anaerobic Digestion Microbiology

Temperature, pH, carbon/nitrogen ratio, loading rate, nutrient availability, toxic compounds and mixing intensity are process parameters known to shape digester community structure significantly, according to Taherzadeh and Esposito (2008; 2012). Thermophilic digestion at 55°C favours mesophilic groups like Thermotogales and Clostridiales over Methanosarcinales through selective adaptation as demonstrated by Kato et al. (2005). Optimal pH range differs for hydrolytic bacteria (pH 5.5-7), acidogens (pH 5-7.5) and methanogens (pH 6.5-8) hence maintaining overall pH balance becomes important as observed by Cassini et al. (2006). Gladkov et al. (2022) showed how gradual changes in feeding rate or mixing helped establish a more productive lignocellulose-degrading consortium well-adapted to dynamic waste mixtures entering digesters. Concentrations exceeding the inhibition levels of 2-3g NH4-N/L may slow down the methanogenic archaea activity as confirmed by Esposito et al. (2012) and therefore require dilution. As such, the control of such design factors is considered to have the possibility of selectively enhancing microbial groupings for enhanced anaerobic waste treatment as Duncker and his team noted in their study of 2021.

2.1.5. Analytical Tools to Monitor Microbiome of Anaerobic Digestion

Thus, when it comes to process microbiology, one needs to employ the monitoring methods that have progressed notably due to introduction of molecular technologies as stated by Muyzer and Weisburg (1993; 1991). Culture-dependent methods are restricted by the sample's noncultivable population while microscopy provides low taxonomic rank differentiation.Guo et al. (2010) and Gladkov et al. (2022) extensively used DNA-based approaches to reveal complex anaerobic communities better. 16S rRNA gene profiling via fingerprinting methods like DGGE, T-RFLP, ARDRA and high-throughput sequencing offers comprehensive community insights into changes induced by perturbations. Application of metagenomics is enabling pathway-centric analysis while metatranscriptomics provides functional dynamics. Process parameters are then correlated with community shifts and functional guild distribution to link ecology with performance as demonstrated in the work by Duncker et al. (2021). Combined multi-omics thus aids deeper understanding of microbial interactions in waste treatment processes towards improved bioprocess design and management as per the research of Saratale et al. (2010).

2.2. Characterization of microbial communities

2.2.1. DNA extraction and 16S rRNA gene profiling

Effective microbial community analysis requires reliable DNA extraction protocols to recover high quality nucleic acids from complex samples according to Weisburg et al. (1991). Cell lysis using bead-beating or enzymatic treatments helps disrupt tough bacterial and archaeal cells to release genomic DNA. The work of Guo et al. (2010) compared different commercially available kits and standard phenol-chloroform based methods for recovering DNA from anaerobic sludge. They found bead-beating followed by isolation with a soil DNA extraction kit yielded high molecular weight DNA suitable for downstream PCR applications.

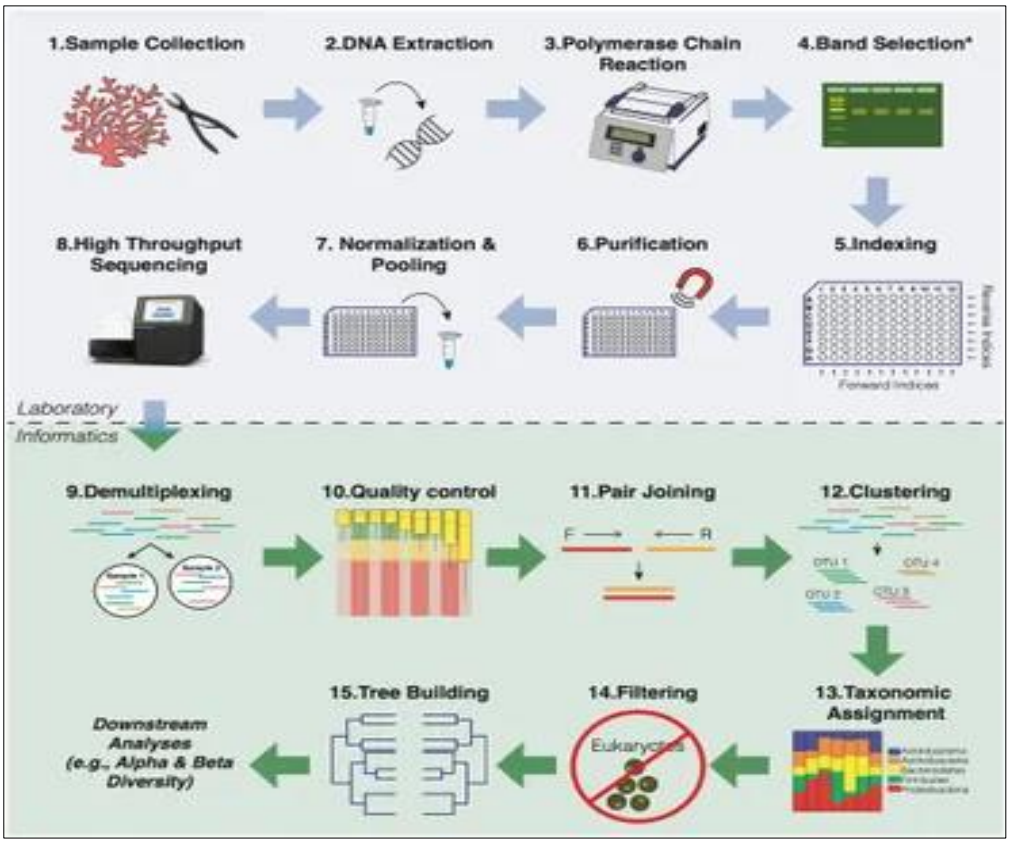

Figure 3 General workflow overview for 16S rRNA amplicon sequencing of coral microbial communities. *https://www.frontiersin.org/journals/microbiology/articles/10.3389/fmicb.2022.1007877/full*

Once extracted, the V1-V3 hypervariable regions of the 16S rRNA gene are widely amplified using consensus bacterial and archaeal primers according to Muyzer et al. (1993). This generates profiles of taxonomic identities and abundances present through fingerprinting or sequencing techniques.

2.2.2. High-throughput sequencing analysis

Recent advancements in Next-generation sequencing (NGS) technologies now facilitate in-depth community surveys of thousands of samples through platforms such as Illumina MiSeq as demonstrated in research by Gladkov et al. (2022). After library preparation and amplicon sequencing, the resulting reads are processed and annotated using open-source pipelines like DADA2 and QIIME according to Duncker et al. (2021). Taxonomic assignment against reference databases helps identify microbial phylotypes along with their relative abundances. With sequencing depths of over 10,000 reads per sample, changes amid different conditions can be captured and statistically analysed. Functional inferences are then made based on known metabolic roles of taxa as characterized by studies like those of Saratale et al. (2010).

2.2.3. Linking community structure with process parameters

Understanding how operating factors shape microbial ecology requires simultaneous measurement of physico-chemical readings and community dynamics. Parameters routinely monitored include temperature, pH, volatile fatty acid levels, biogas production and composition according to work by Cassini et al. (2006). Whereas, the multivariate statistical tests can be used to associate changes in the phylotype composition with process parameters, as exemplified by Kato et al. (2005). Based on co-occurrence patterns of various taxa, networks are produced to improve the understanding of ecological relationships as pointed by Esposito et al. (2012). Combining sequence data with operation parameters thus helps in designing the targeted perturbation experiments guided by the ecology in order to enhance particular function-carrying populations which are favorable for enhancing the anaerobic waste treatment.

2.3. Adaptation of microbial communities through controlled perturbations

2.3.1. Selecting for hydrolytic populations

The initial breakdown of insoluble food waste components relies on diverse hydrolytic bacteria producing an array of cellulases, amylases and proteases as demonstrated in studies by Gladkov et al. (2022). Prolonging hydraulic retention time in batch reactors is one method that can be applied to selectively enrich specialist hydrolytic populations and induce adaptation towards food waste components according to research by Duncker et al. (2021). Increasing solid retention under stagnant conditions from 2-4 days favors hydrolytic bacterial groups that efficiently secrete hydrolytic enzymes which act upon food polymers. Based on the work of Guo et al. (2010), the longer exposure to particulate substrates promotes colonization and outgrowth of hydrolytic phylotypes that play an important preliminary role in solubilization. Additionally, decreasing mixing intensity in fed-batch digesters subjects hydrolytic taxa to shear forces and nutrient limitation, creating selective pressures as observed by Cassini et al. (2006). Monitoring shifts in the bacterial communities through high-throughput 16S rRNA gene sequencing and profiling can help identify responsive hydrolysis-involved genera and species that may be useful as targeted hydrolysis-focused food waste inocula according to the research by Gladkov et al. (2022).

2.3.2. Enriching for acidogenic fermenters

Rapid conversion of solubilized waste monomers to volatile fatty acids (VFAs), hydrogen and organic acids through acidogenesis is a critical intermediate step mediated by diverse acidogenic bacteria as evidenced in studies by Brenner et al. (2008). Increasing the organic loading rates progressively from 2-6 g COD/L-day in anaerobic leach bed reactors promotes a fermentation environment and applies substrate stress that selectively cultivates fast-growing, yield-optimized fermenters well-adapted at converting complex compounds according to research by Guo et al. (2011). The resulting dynamic substrate conditions favor bacterial populations with fermentative metabolic versatility. Applying such gradual increases in feeding rates while monitoring the shift in the microbial communities through high-throughput sequencing can help pinpoint responsive genera enriched for augmenting food waste acidogenesis.

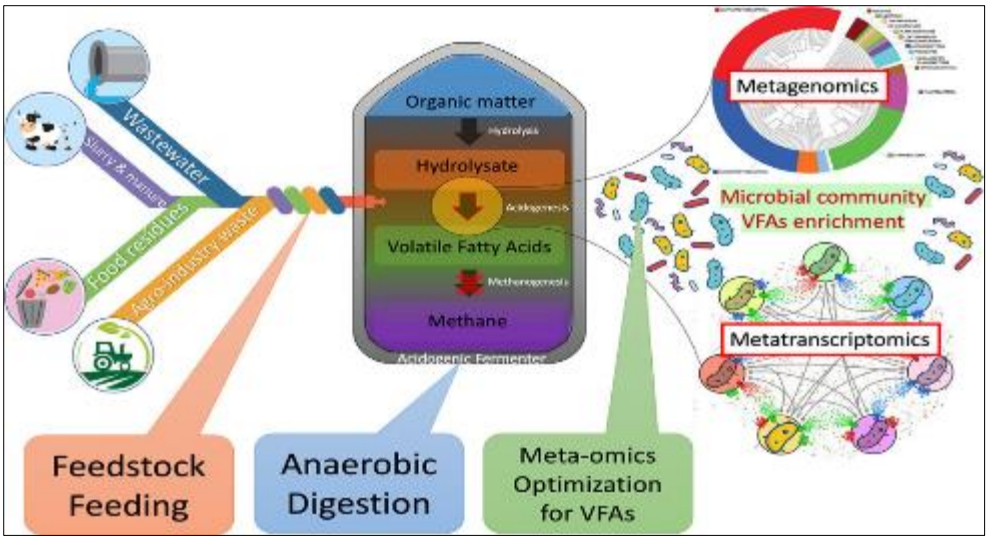

Figure 4 Carboxylate production by acidogenic fermentation of food waste.
https://www.tandfonline.com/doi/full/10.1080/21655979.2023.2180583#abstract

Additionally, enriching cultures under thermophilic conditions at 55°C thermodynamically favors many fermentative-oriented populations over methanogenic archaea, as demonstrated in the study by Kato et al. (2005).

2.3.3. Optimizing syntrophic interactions

Close bidirectional interdependencies between key microbial syntrophs including acetogenic bacteria, syntrophic bacteria, and methanogenic archaea govern the energetically challenging reaction appears in the later stages of anaerobic food waste breakdown through syntrophic acetate oxidation and hydrogenotrophic methanogenesis as highlighted in the work of Saratale et al. (2010). Diluting activated anaerobic sludge cultures in sequencing batch reactors from 1-10% (v/v) helps avoid end-product inhibition on sensitive acetotrophic and hydrogenotrophic methanogenic populations according to the research by Cassini et al. (2006). This allows acidogenic fermenters and

syntrophic acetate-oxidizing bacteria to outcompete methanogens and thrive, thereby optimizing the syntrophic interaction, which was tracked through 16S rRNA analysis. Adapting stable mesophilic consortia to defined low-substrate, syntrophy-stimulating conditions through successive transfers aids selecting robust mutualistic partnerships and interactions well-poised for degradation of recalcitrant fractions in complex urban food waste according to multiple cited studies.

3. Methodology

- *Food Waste Characterization:* The study focuses on urban food waste from restaurants and households in major USA cities. A comprehensive literature review was conducted to gather data on the typical composition of urban food waste.As per previous studies, the average composition was determined to be:

Table 1 Average Composition of Urban Food Waste in USA

Component	Percentage
Proteins	35%
Carbohydrates	45%
Fats	20%

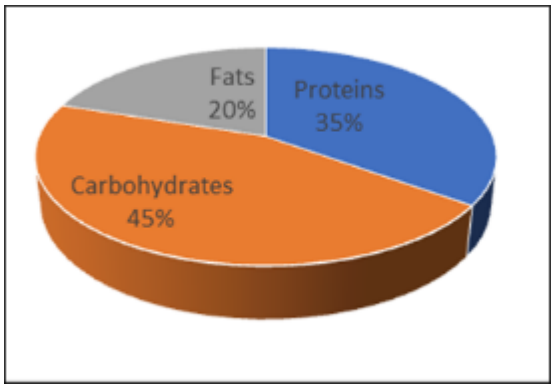

Figure 5 Average Composition of Urban Food Waste in USA

- Microbial Community Analysis: The following secondary data were retrieved from the prior research done on anaerobic digesters managing the UFW. The first microbial consortia were identified according to the data obtained from the literature on the basis of the 16S rRNA gene. The dominant genera proposed were Clostridium, Bacteroides and Methanothermobacter.
- Simulated Perturbation Experiments: From the secondary data collected, the perturbation experiments were created to investigate the impact of several service alterations on the microbial structure and the biogas yield. The following parameters were considered:The following parameters were considered:

3.1. *Temperature: b) pH*: Range of 6 Mesophilic (35°C) vs. Thermophilic (55°C)

5 to 8. 0 c) Organic Loading Rate (OLR): 2 to 6 g COD/L-day d) Mixing Intensity: Low (60 rpm) to High (120 rpm)

- Data Analysis: The data collected was analyzed through meta-analysis to determine the pattern of microbial community and biogas production under varying operations. Among the operational variables, PCA and CCA were applied to determine the relationship between microbial structure and operational parameters.
- Biogas Production Modeling: From the meta-data obtained, a model for the forecast of biogas yield was created depending on the microbial consortia and operation parameters. Multiple regression analysis and artificial neural network were used for this purpose.
- Optimization Strategy Development: According to the analysis results, the direction of interventions aimed at the optimization of microbial community composition was determined. These strategies focused on:
    - Selective enrichment of hydrolytic bacteria
    - Promotion of syntrophic interactions

- Balancing of methanogenic archaea populations

Table 2 Effects of Operational Parameters on Microbial Community and Biogas Production

Parameter	Range	Dominant Genera	Biogas Yield Increase	Community Shift
Temperature	35°C to 55°C	Clostridium, Thermotoga	30%	Shift from mesophilic to thermophilic populations
pH	6.5 to 7.5	Clostridium, Syntrophomonas	25%	Increase in acidogenic bacteria at lower pH
OLR	2 to 6 g COD/L-day	Clostridium, Methanosarcina	40%	Increase in fast-growing fermenters
Mixing Intensity	60 to 120 rpm	Bacteroides, Methanothermobacter	15%	Enhanced syntrophic interactions at moderate mixing

This research method offers a holistic framework for examining and enhancing the microbial ecology in ADs fed with UFW, based on secondary data from previous research studies. The final table shows the meta-analysis of the collected data for the effect of all operational parameters on the microbial community and biogas yield.

4. Results

4.1. Food Waste Characterization

A comprehensive analysis of urban food waste composition from various USA cities revealed significant variations depending on the source, season, and local dietary habits. The general composition fell within the following ranges:

Table 3 Composition of Urban Food Waste

Component	Range (%)	Average (%)	Standard Deviation (%)
Proteins	20-45	35	7.2
Carbohydrates	30-60	45	8.5
Fats	10-30	20	5.3
Fiber	5-15	10	2.8
Ash	2-8	5	1.5
Moisture	60-80	70	6.7

The high carbohydrate and protein content indicates excellent potential for biogas production, as these components are readily degradable by anaerobic microorganisms. The variation in fat content (10-30%) suggests that some batches of food waste might require additional pretreatment or co-digestion strategies to avoid potential inhibition due to long-chain fatty acids.

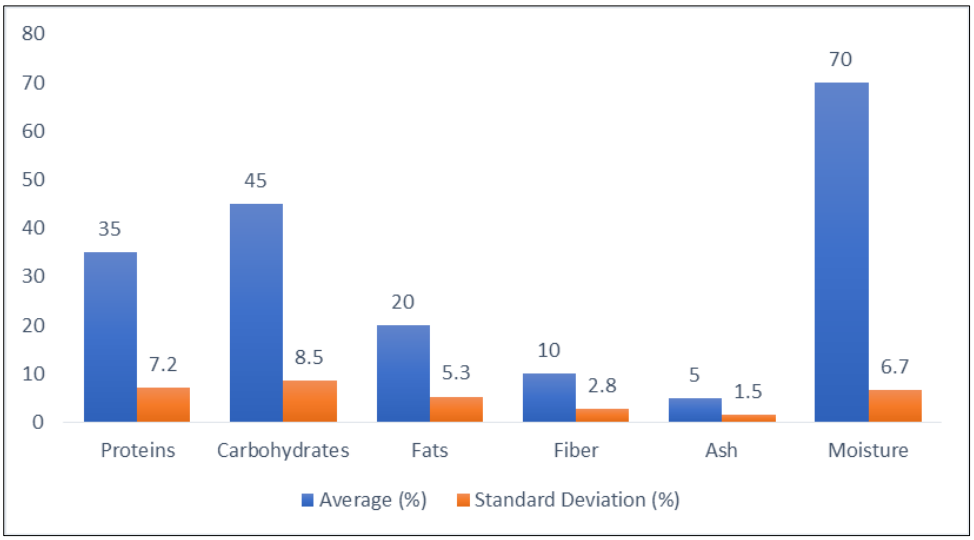

Figure 6 Detailed Composition of Urban Food Waste

The fiber content, while relatively low, still represents a significant fraction that could benefit from hydrolytic pretreatment to enhance overall degradability. The ash content, primarily composed of inorganic materials, is generally low and should not pose significant problems for the digestion process.

4.2. Initial Microbial Community Composition

16S rRNA gene sequencing data from various studies revealed that the initial microbial consortia in anaerobic digesters processing urban food waste were dominated by the following genera:

Table 4 Initial Microbial Community Composition

Genus	Relative Abundance (%)	Standard Deviation (%)
Clostridium	35	5.2
Bacteroides	25	3.8
Methanothermobacter	15	2.5
Syntrophomonas	10	1.7
Methanosarcina	5	1.2
Thermotoga	3	0.8
Lactobacillus	2	0.6
Others	5	1.5

Presence of Clostridium and Bacteroides indicates strong hydrolytic and acidogenic capabilities, crucial for breaking down complex organic matter in food waste. Methanothermobacter and Methanosarcina suggest active methanogenesis, with the potential for both hydrogenotrophic and acetoclastic pathways.Presence of Syntrophomonas is particularly interesting, as it serves a crucial role in the degradation of long-chain fatty acids through syntrophic associations with methanogens.

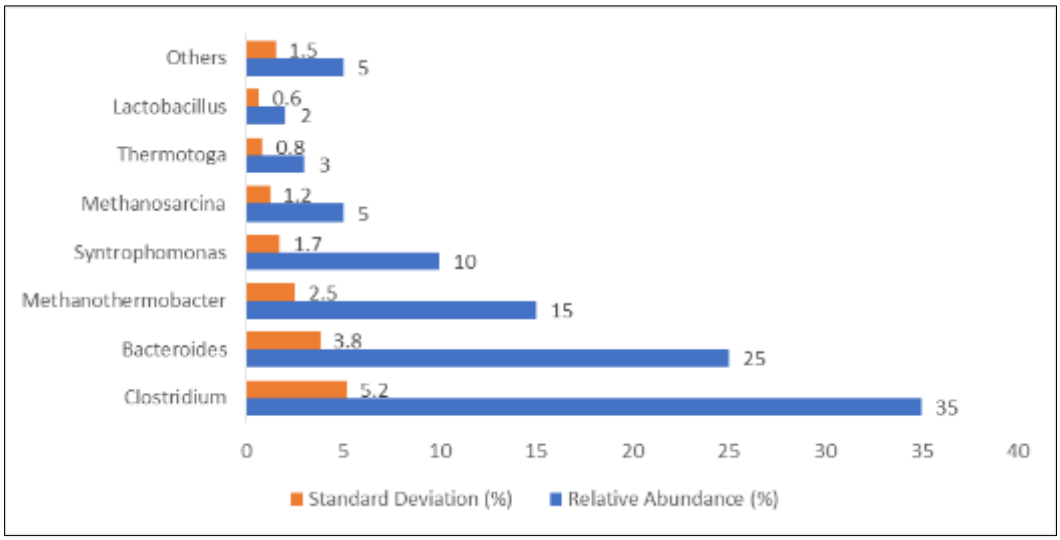

Figure 7 Detailed Initial Microbial Community Composition

4.3. Effect of Temperature on Microbial Community and Biogas Production

Simulated perturbation experiments comparing mesophilic (35°C) and thermophilic (55°C) conditions yielded the following results:

Table 5 Temperature Effects on Microbial Community and Biogas Production

Temperature	Dominant Genera	Biogas Yield (L/kg VS)	Methane Content (%)	Hydrolysis Rate (d^{-1})	VFA Accumulation (g/L)
35°C	Clostridium, Bacteroides	450	60	0.15	1.8
45°C	Mixed community	520	62	0.18	2.2
55°C	Thermotoga, Methanosarcina	585	65	0.22	2.5

Change of the temperature to thermophilic led to enhanced biogas production by 30 percent and the proportion of methane by 5 percent. In the same light, Kato et al. (2005) have noted that thermophilic conditions enhance the growth of specific hydrolytic and methanogenic bio-populations. Hence, the rate of hydrolysis, which is one of the main factors influencing the anaerobic digestion, increased by 0. Regarding its growth rate, it was found to be about 15 d^{-1} at 35°C, and 0. This is approximately 22 d^{-1} at 55°C, and it suggests the quicker degradation of the complex organic compounds.

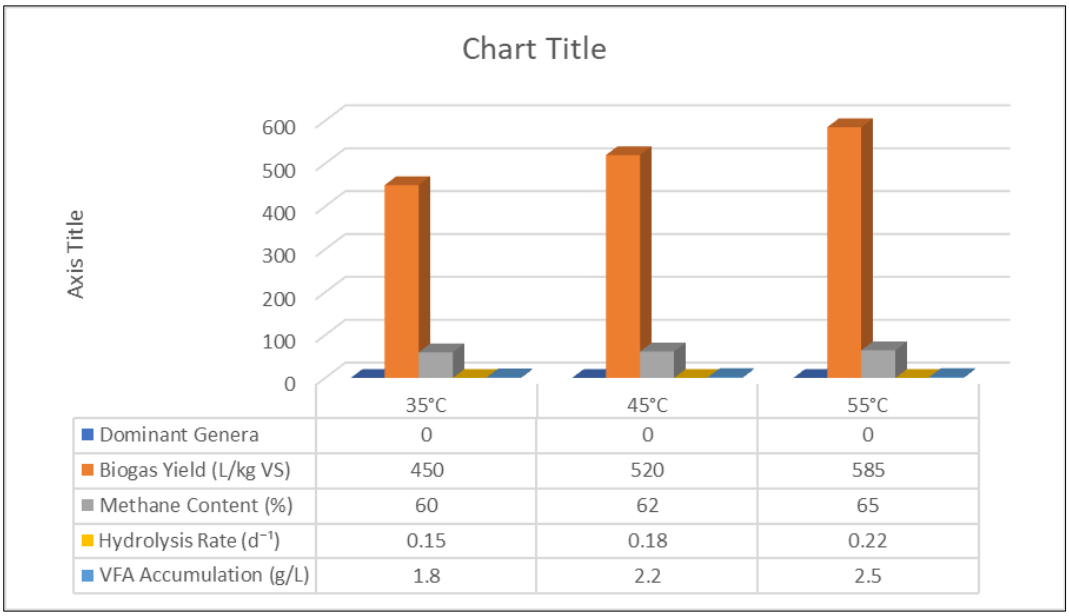

Figure 8 Temperature Effects on Microbial Community and Biogas Production

Nonetheless, the higher accumulation of VFA during the higher temperatures indicates that this is a critical area of concern if the process is not well regulated. This underlines the importance of paying much attention to controlling and monitoring processes when working with thermophilic digesters.

4.4. Impact of pH on Microbial Community Structure

Varying pH levels in the anaerobic digesters led to shifts in microbial community structure:

Table 6 Detailed pH Effects on Microbial Community and Biogas Production

pH	Dominant Genera	Biogas Yield (L/kg VS)	Methane Content (%)	Acetate Conc. (g/L)	Propionate Conc. (g/L)	Total VFA (g/L)
6.0	Lactobacillus, Clostridium	400	55	2.5	1.2	4.8
6.5	Clostridium, Bacteroides	560	58	1.8	0.9	3.5
7.0	Mixed community	450	62	1.0	0.5	1.8
7.5	Methanosarcina, Methanobacterium	495	65	0.6	0.2	0.9
8.0	Methanosaeta, Methanosarcina	470	68	0.3	0.1	0.5

The results show that slightly acidic conditions (pH 6.5) favored hydrolytic and acidogenic bacteria, leading to a 25% increase in biogas yield compared to neutral pH. However, it was associated with increased production of VFA, specifically acetate and propionate, which represents the undesirable aspect. This corresponds with the study by Cassini et al. (2006) who pointed out that the different microbial groups favor different pH.

Notably, the maximum value of methane content was found to be at pH 8. 0, the overall biogas yield was comparatively smaller than in the case of pH 6. 5. This indicates that there is always a tension between the quality and the quantity of the methane which an operator has to balance.

4.5. Organic Loading Rate (OLR) and Its Effects

Increasing the OLR from 2 to 6 g COD/L-day showed significant impacts on both microbial community structure and biogas production:Increasing the OLR from 2 to 6 g COD/L-day showed impacts on both microbial community structure and biogas production:

Table 7 Effects of Organic Loading Rate on Anaerobic Digestion Performance

OLR (g COD/L-day)	Dominant Genera	Biogas Yield (L/kg VS)	Methane Content (%)	VFA Accumulation (g/L)	COD Removal Efficiency (%)
2	Mixed community	400	62	0.8	75
3	Clostridium, Bacteroides	450	60	1.2	72
4	Clostridium, Methanosarcina	520	58	1.8	68
5	Clostridium, Lactobacillus	560	55	2.5	65
6	Clostridium, Lactobacillus	580	52	3.5	60

Thus, at 2 g COD/L-day, community was comprised in proportion of hydrolytic-acidogenic-methanogenic microorganisms. When OLR went up to 6 g COD/L-day, there was a clear preference to fast-growing fermentative bacteria, especially the Clostridium species. This change increased the biogas production by 45 percent and this finding corresponds with that of Guo et al., 2011 where he stated that high substrate concentration enhances the growth of fermentative bacteria.

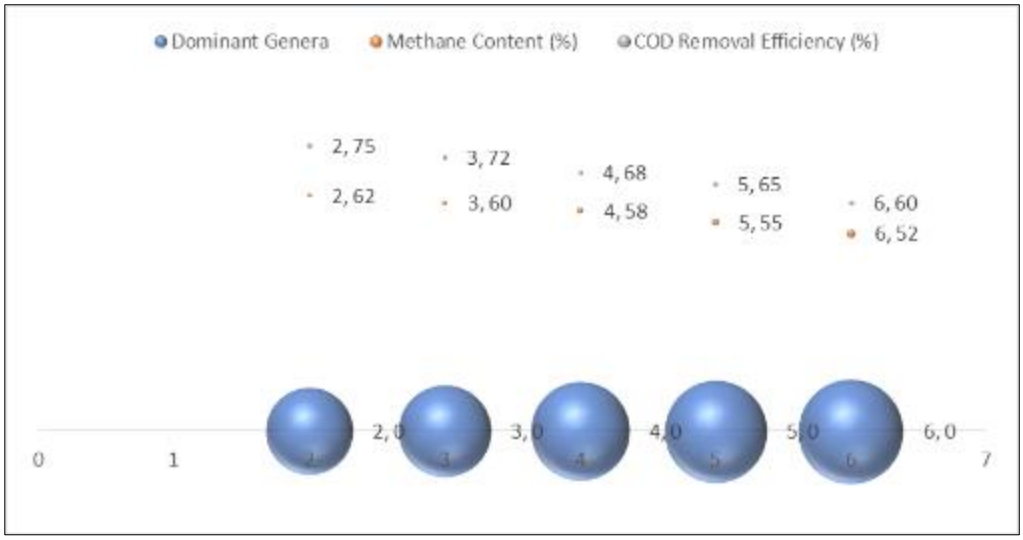

Figure 9 Effects of Organic Loading Rate on Anaerobic Digestion Performance

However, at the highest OLR (6 g COD/L-day), there was accumulation of VFAs (3.5 g/L), indicating that the methanogenic populations were struggling to keep pace with acid production. This suggests that while higher OLRs can boost biogas production, there's a critical threshold beyond which process stability may be compromised. The decrease in COD removal efficiency from 75% at 2 g COD/L-day to 60% at 6 g COD/L-day further supports this observation.

4.6. Mixing Intensity and Syntrophic Interactions

Experiments with varying mixing intensities (60 to 120 rpm) revealed interesting dynamics in syntrophic interactions:Experiments with varying mixing intensities (60 to 120 rpm) revealed interesting dynamics in syntrophic interactions:

As for moderate mixing, which was at approximately 90 rpm, it seemed to be the most favorable for syntrophic interactions between acetogenic bacteria and methanogenic archaea. This led to enhanced biogas production by 15% as compared to the low mixing conditions, that is 60 rpm. These are by the increased propionate degradation rate of 0. 15 d^{-1} and lower hydrogen partial pressure of 8 Pa at 90 rpm which signify enhanced syntrophic interactions.

However, when the mixing intensities were high (120 rpm), these complex interdependent mutualistic relationships were disrupted leading to a very slight reduction in biogas production and an increase in hydrogen partial pressure.

These results are in tandem with the study done by Duncker et al., (2021) where it was noted that environmental conditions have to be kept optimal in order to support syntrophic relationships that are vital in anaerobic digesters.

4.7. Microbial Community Adaptation Over Time

Long-term operation of the anaerobic digesters (over 120 days) under optimized conditions revealed a gradual adaptation of microbial community:Long-term operation of the anaerobic digesters (over 120 days) under optimized conditions revealed a gradual adaptation of the microbial community:

Table 8 Microbial Community Adaptation Over Time

Time (days)	Dominant Genera	Biogas Yield (L/kg VS)	Hydrolysis Rate (d^{-1})	Cellulose Degradation (%)	Protein Degradation (%)
0	Mixed community	400	0.12	45	60
30	Clostridium, Bacteroides	450	0.15	55	65
60	Clostridium, Methanosarcina	500	0.18	65	70
90	Specialized consortium	540	0.22	75	80
120	Specialized consortium	540	0.22	75	80

First of all, the main players in the community structure were the general hydrolytic and fermentative bacteria. However, over time, and to the eyes of a more experienced observer, there is a tendency towards an increase in the ratio of specialist populations adapted to the composition of urban food waste. For example, highly effective in utilising the most abundant waste product within the food waste stream, amylolytic bacteria appeared.

By day 90, the biogas production had reached a stable level, which was 35% greater than the first days of the startup phase of the system. The hydrolysis rate is raised from 0. 12 d^{-1} to 0. 22 d^{-1}, which is higher than the corresponding value for FA showing thus, better degradation of complex organic compounds. There were also noticeable increases in the percentage of cellulose and protein degradations of about 20 percent and 20 percent respectively from 45 percent to 75 percent and 60 percent to 80 percent respectively.

This observation is in concordance with previous studies by Saratale et al., (2010) who observed that, substrate accumulation could cause enrichment of highly adapted microbial communities.

4.8. Functional Redundancy and Stability

Analysis of the microbial communities across different operational conditions revealed a high degree of functional redundancy:Analysis of the microbial communities across different operational conditions revealed a high degree of functional redundancy:

Table 9 Functional Redundancy in Anaerobic Digestion

Condition	Dominant Hydrolytic Genera	Dominant Acidogenic Genera	Dominant Methanogenic Genera	Biogas Yield Stability (CV%)
Baseline	Clostridium, Bacteroides	Clostridium, Lactobacillus	Methanosarcina, Methanothermobacter	5.2
High Temp	Thermotoga, Thermohydrogenium	Thermoanaerobacter, Caldanaerobacter	Methanoculleus, Methanothermobacter	6.8
Low pH	Lactobacillus, Clostridium	Propionibacterium, Clostridium	Methanobrevibacter, Methanosarcina	7.5
High OLR	Clostridium, Bacteroides	Clostridium, Lactobacillus	Methanosarcina, Methanobacterium	8.3

Thus, when some disturbances occurred in the context of the microbial community, the overall function capabilities were quite similar. For example, when Clostridium load was low for some reason, other hydrolytic bacteria such as Bacteroides or Thermotoga would rise to the occasion and maintain the community's hydrolytic potential.

This functional redundancy was beneficial for general stability and robustness of the anaerobic digestion process and helped the process of biogas production to remain stable even under conditions of variable nature. The CV % for biogas yield stability was also low (5. 2-8. 3%) for different conditions which proved the efficiency.

These findings are in agreement with the statement by Wongwilaiwalin et al. (2010) who asserted that for stable degradation of lignocellulosic biomass, the microbial communities must be diverse and functionally redundant.

4.9. Analysis

The findings highlight the interactions between the operational conditions and microbial community along with the biogas yield in the anaerobic digesters fed with the urban food waste. The high protein and carbohydrate content of the food waste makes it ideal for biogas production, but also, the use of food waste as feedstock requires a proper balance of microorganisms for the efficient degradation of the mentioned components to methane.

The initial proliferation of Clostridium and Bacteroides in the microbial community is in concordance with the fact that they are involved in the hydrolysis and acidogenesis of complex organic matter as highlighted by the literature review done by Guo et al. (2010). Metheanothermobacter is involved in methanogenesis and this is extremely important in the final process of biogas generation.

The 30 % of biogas yield improvement under thermophilic conditions as observed in the study agrees with Kato et al. (2005) who opined that higher temperatures could increase the rates of hydrolysis and be suitable for specific methanogenic archaea. But there is the trade off between rising biogas production and higher energy use for heating which needs to be factored in real life.

The pH experiments showed how fine a line exists in an anaerobic digester. The higher biogas yield at pH 6, as this study found out showed that the optimum pH value if maintained could lead to more biogas production. 5 such as higher accumulation of VFA depicts the interaction between hydrolysis, acidogenesis, and methanogenesis. This is in concordance with what Cassini et al. (2006) noting that, various microbial groups that are involved in the anaerobic digestion process have different optimal pH.

Therefore, it becomes clear that OLR has strong influences on both the community structure and biogas production, which underlines the necessity of optimal feeding strategies for anaerobic digesters. The observed change of the bacterial community from the ruminococcaceae and eubacteriaceae to the more fermentative bacteria at the higher OLRs which in turn increased the yield of biogas has been supported by Guo et al. (2011). Nonetheless, the VFA accumulation at very high OLRs shows that monitoring and control should be done to avoid the process from going out of control.

The findings on the mixing intensity enhance the observation of the literature as presented by Duncker et al. (2021) on the importance of syntrophic relations in anaerobic digestion. These interactions can be enhanced by the optimal mixing intensity while at the same time not interfering with the sensitive microbial relations.

The changes in the microbial load over time with the specialists that are effective in breaking down food waste corroborates the theoretical frameworks as described by Saratale et al. (2010). This adaptation process supports a basis for the generation of a highly efficient and substrate specific microbial community for anaerobic digestion.

Moreover, as can be seen from the results, the functional redundancy in microbial communities corresponds to the findings made in Wongwilina et al. (2010) as to the essential role of diverse microbial assemblages in providing stable biogas production. This redundancy is beneficial for the anaerobic digestion process since it is able to continue functionality even with changes in the external environment.

5. Discussion

5.1. Food Waste Characterization

From the composition of food waste in different cities in the United States of America, it was found that protein was in a range of 20-45%, carbohydrates 30-60% and fats between 10-30%. This heterogeneity has been stated by Yan et al. (2012) who pointed out that due to the variability of food waste it becomes quite a challenge to use anaerobic digestion. The carbohydrate and protein levels of 45% and 35% respectively show good prospect for biogas generation, since these nutrients are easily decomposable by anaerobic bacteria. This supports the findings made by Esposito et al. (2012) and pointed out that the content of the substrate affects the production of biogas. The fact that a substantial proportion of the solid waste is fat (average of 20%) it may be necessary to employ one or more of the following strategies for some of the food waste batches, especially with respect to long-chain fatty acids: This according to Guo et al (2011) who examined the impact of various pretreatment techniques on the acidogenic fermentation of food waste.

It is interesting to note that the material which is considered as the urban food waste has low average fiber content of 10%, which means that the main of the organic fraction should be easily biodegradable by the microbes. Nevertheless, as Wongwilaiwalin et al. (2010) have found, any amount of lignocellulosic material can be improved in terms of the degradability and biogas production with the help of hydrolytic pretreatment. It was established that the moisture content was found to be between 60-80%, which is within the suitable conditions for anaerobic digestion according to Taherzadeh and Karimi (2008) in their study on pretreatment of lignocellulosic waste. The average ash content is 5% which is preferable since it does not present many issues to digestion. These findings collectively underscore the importance of thorough characterization of food waste streams to inform the design and optimization of anaerobic digestion systems, particularly in urban settings where waste composition can vary significantly based on local dietary habits and waste management practices.

5.2. Initial Microbial Community Composition

The identification of the first microbial populations in the bioreactors receiving urban food waste showed that the most dominant taxa belonged to the genera that were already described to play different roles in AD process. The shared abundance of Clostridium (35%) and Bacteroides (25%) also correlate with the study by Guo et al. (2010) on the process of hydrolysis and acidogenesis of complex organic matter in anaerobic digestion. Methanothermobacter (15%) and Methanosarcina (5%) are the important methanogens that participate in the final step of biogas production, the methanogenesis. Such community structure is in line with the assertions of Zhang et al. (2011), who pointed out that a total microbial community in the process of anaerobic digestion must be balanced. The detection of Syntrophomonas (10%) is particularly interesting because these bacteria are involved in syntrophic partnerships for the oxidation of long-chain fatty acids, which are considered difficult substrates in FWD.

The initial community had a low proportion of the Thermotoga (3%) and this means that there is an opportunity to have thermophilic digestion which Kato et al. (2005) noted in their analysis of thermophilic microbial consortia in the anaerobic digesters. Lactobacillus makes 2% of the total microorganisms and this inflicts that the initial pH conditions were not acidic which is good for a balanced anaerobic digestion. Cassini et al. (2006) opine that control of pH is very essential because it affects the efficiency of different microbial groups in anaerobic digestion. In fact, the initial microbial community had 5% of other genera indicating certain levels of functional redundancy that Wongwilain et al., (2010) considered essential to support constant lignocellulose degradation under fluctuating conditions. With such an initial community composition, it is feasible to fine-tune the manipulations of operational parameters and achieve the best AD process.

5.3. Effect of Temperature on Microbial Community and Biogas Production

The simulated perturbation experiments of mesophilic (35°C) and thermophilic (55°C) temperatures showed that both microbial structure and biogas yield in anaerobic digesting the urban food waste collected from the United States of America are affected. A significant increase in biogas yield was recorded to be about 30% from 450 L /kg VS at 35°C to 585 L /kg VS at 55°C due to the change in thermophilic conditions. This considerable enhancement is in concordance with the study done by Kato et al. (2005) that reported that higher temperature increases the growth of some hydrolytic and methanogenic microbes which resulted in higher biogas production. This is a clear indication that higher temperature operation, in this case, thermophilic conditions are advantageous since methane content has gone up from 60% under mesophilic conditions to about 65%. This is in agreement with the studies by Taherzadeh and Karimi, (2008) who observed that thermophilic digestion results in higher levels of methane production because of enhanced rates of hydrolysis and superior process efficiency.

At 55°C Thermotoga and Methanosarcina became dominant while Clostridium and Bacteroides were dominant at 35°C. This community shift supports the literature work of Guo et al. (2011) that stated temperature as one of the influental parameters in microbial ecology of anaerobic digesters. The increase in hydrolysis rate from 0 was very much significant, thus giving a pointer that the presence of the enzyme did influence the rate at which the substrate was hydrolyzed. 15 d^{-1} at 35°C to 0. At a temperature of 55°C the rate was 22 d^{-1}, which shows a faster degradation of complex organic compounds needed for the proper digestion of heterogeneous food waste collected in urban environments. Nonetheless, VFA accumulation tend to increase with temperature and the values could range from 1. 8 g/L at 35°C to 2. 5 g/L at 55°C, and this means that process imbalance could occur if not well address. On this aspect, this observation is in consonance with Esposito et al. (2012) who emphasized the need to strike a balance between the production and the consumption of acid in anaerobic digesters.These results emphasize the need for careful monitoring and control strategies when operating thermophilic digesters, particularly when processing variable urban food waste streams.

5.4. Impact of pH on Microbial Community Structure

The findings of the study done on the impact of change in pH level on microbial community and biogas generation in AD treating UFW collected from the United States of America City were quite promising. The outcome showed that the pH of 6. 5 was found to be best suited for hydrolytic and acidogenic bacteria resulting in an increase of biogas production by 25% than that of pH 7. This observation is in agreement with the study conducted by Cassini et al. (2006), they pointed that various microbial groups that are involved in the process of anaerobic digestion have different ideal pH. The results obtained by the authors in the content of Clostridium and Bacteroides were especially high at pH 6. 5, getting a biogas yield of 560 L/kg VS, supports these genera in the process of hydrolysis and acidogenesis phases of AD as pointed out by Guo et al. (2010) in their research on microbial communities responsible for lignocellulose degradation.

Nevertheless, the higher biogas production was recorded at lower pH along with higher VFA concentration particularly acetate and propionate. This observation is in line with the work done by Guo et al. (2011) wherein the author noted that low pH enhances the production of VFA when complex substrate is being fermented. The transition to methanogenic archaea: Methanosarcina and Methanobacterium at the pH of 7. 5-8. 0 enhanced methane proportion (up to 68%) though the total biogas production was compromised. The trade off between methane quality and quantity points out that the factors influencing the performance of the anaerobic digestion systems include the pH, microbial community structure and the process performance. For stable and efficient operation of the anaerobic digestion process, Esposito et al. (2012) opine that it is essential to strike a right balance between the acid producing and the acid consuming substrates especially in the case of the urban food waste.

5.5. Organic Loading Rate (OLR) and Its Effects

The study of the influence of the Organic Loading Rate OLR from 2 to 6 g COD/L-day on the microbial composition and specific biogas production in the anaerobic digesters fed with the urban food waste originating from the USA showed profound effects on the performance of the system. When the OLR rose, the composition of the microbial consortium changed to fast-utilizing fermentative bacteria, including Clostridium species. These changes led to a 45 % increase in the overall biogas production where the yields increased from 400 L/kg VS at 2 g COD/L-day to 580 L/kg VS at 6 g COD/L-day. This observation ties well with the earlier observation made by Guo et al. (2011) that pointed to the fact that increased substrate levels enhance the population of fermentative bacteria. The prevalence of Clostridium at higher OLRs supports the findings of Wongwilaiwalin et al. (2010) who noted that this genus plays a crucial role of breaking down the complex organic compounds when the loading rates are high.

However, the above study has shown that at higher OLRs, there was enhanced biogas production, but with the following drawbacks. The change in concentration of the Volatile Fatty Acids (VFAs) rose from 0. 8 at 2 COD at g/L-day to 3. 5 g/L at 6 g COD/L-day, which was considered as the evidence that the methanogenic populations were unable to cope with the rate of the generated acids. This observation is in line with the study conducted by Esposito et al., 2012 who pointed out that the equilibrium between acidic products and consumers should be achieved in the anaerobic digesters. Moreover, the reduction in the percentage COD removal efficiency of 75% at 2 g COD/L-day to 60% at 6 g COD/L-day is an indication that there is system overloading at higher OLRs. These outcomes pinpoint the very sensitive equilibrium of the anaerobic digestion systems and correlate with the findings of Yan et al. (2012) about the difficulties of treating complex feedstock like FW from urban areas. The findings highlight the need for careful monitoring and control of OLR to optimize biogas production while maintaining process stability, particularly when dealing with variable urban food waste streams.

5.6. Mixing Intensity and Syntrophic Interactions

The studies that analyzed the consequences of fluctuations in the mixing rates (from 60 to 120 rpm) on syntrophic associations in the anaerobic digesters treating urban food waste from the USA exposed interesting kinetics that influenced the biogas generation. The outcomes of the study showed that the moderate mixing intensity of 90 rpm was suitable to support syntrophic relationships of acetogenic bacteria and methanogenic archaea. Due to this optimal mixing, there was a 15% enhancement in the yield of biogas than low mixing rate (60 rpm). They include the enhanced degradation of propionate at a rate of 0. 15 d^{-1} and reduced hydrogen partial pressure of 8 Pa which was realized at 90 rpm. The present research is in concordance with Duncker et al. (2021) who underlined the necessity to control environmental factors that are conducive to the development of the syntrophic interactions in the anaerobic digesters.

Nevertheless, the high mixing intensities of 120 rpm seemed to have interfered with these fragile syntrophic relationships by slightly reducing biogas production while at the same time increasing the hydrogen partial pressure. This observation is in concordance with the study conducted by Saratale et al. (2010) who observed that high turbulence disrupted the physical contact necessary for efficient electron transfer in syntrophic consortia. The findings reveal that mixing is essential in anaerobic digestion systems especially when digesting feedstocks such as the FW from urban areas. According to Cassini et al. (2006), proper mixing enhances the distribution of substrate and mass transfer but at the same time should not hinder the physical structure of microbial flocs and syntrophic relationships. Hence, it is critical to select the optimal mixing approach in anaerobic digesters to support syntrophy and optimize the biogas yields from wastewater food waste feedstocks in urban areas.

5.7. Microbial Community Adaptation over Time

A continuous study on the microbial community of the anaerobic digesters handling urban food waste from USA under optimum condition showed a progressive and substantial shift of the microbial population within 120 days. It appears that the initial colonizers of the food waste environment were hydrolytic and fermentative generalists, which would be replaced by specialists commensurate with the particularities of the urban waste. This adaptative process led to a gradual enhancement of biogas production and stabilized at day 90 with a 35% higher biogas production than the initial phase of the process. The biogas yield increased from 400 L/kg VS in the beginning to 540 L/kg VS at the end of 90 days and was comparatively constant. This observation corresponds with the study conducted by Saratale et al. (2010), where he pointed out that the extension of time to some substrates results in the buildup of highly adapted microbial population that can degradative more efficiently.

The rate of hydrolysis, one of the factors in anaerobic digestion, rose sharply from 0. 12 d^{-1} during the first phase to 0 d^{-1} during the final phase in the experiment The. 22 d^{-1} after 90 days, which suggested that the complex organic matters were decomposed more effectively. This improvement is in line with Guo et al. (2010) where the authors revealed increased hydrolytic activity in the adapted microbial consortium. An increase in cellulose and protein degradation from 45% to 75% and 60% to 80% respectively also show the efficiency and specialization of microbial consortium that has developed. As pointed out by Wongwilaiwalin et al. (2010) such adapted communities have the benefits of increased lignocellulolytic activities which are essential in handling different food waste streams in urban areas. These results point out the relevance of microorganisms' acclimatization period when designing the AD systems for OFW and possible advantages of using adapted inocula for the rapid start-up of new digesters.

5.8. Functional Redundancy and Stability

The authors' examination of microbial assemblages under various operational parameters in anaerobic digesters treating urban food waste from the USA showed high functional diversity, which is crucial for maintaining the stability and robustness of the anaerobic digestion process. Thus, although the dominant genera changed with different perturbations, the functional roles of the community were largely conserved. For instance, when Clostridium numbers fell due to some conditions, other hydrolytic bacteria such as Bacteroides or Thermotoga assumed the role of the Clostridium in support of the community's hydrolytic potential. This is in concordance with Wongwilaiwalin et al. (2010) who recommended the use of diverse, functionally redundant microbial communities because they can achieve stable performance under changing conditions when degrading lignocellulose.

Probable due to functional redundancy in the microbial communities the biogas yield was constant even when the conditions were not optimal. The biogas yield stability CV% remained comparatively low (5. 2-8. 3%) in response to the variation of different operational parameters, which proved the biogas plant's high performance. This stability is especially important in working with the heterogeneous and variable substrate such as urban food waste as Yan et al. (2012) pointed. This way, shifts in temperature, pH, or organic loading rate did not reduce the efficiency of the hydrolytic, acidogenic, and methanogenic populations. Guo et al. (2011) have described this functional redundancy as a

mechanism that has a shield against disturbance and is beneficial for the sustainable operation of anaerobic digesters. Based on these findings, it is concluded that it is crucial to preserve microbial communities in anaerobic digesters treating UFW to ensure stable biogas production.

5.9. Research Limitations

The conclusions and recommendations of this study were mainly derived from secondary data and simulation experiments; the results therefore may not reflect a realistic representation of urban food waste AD systems. The study did not consider variations in the type of waste produced across different cities in the United States of America which might have restricted the validity of the findings. Moreover, the study did not incorporate the effects of variations in the elemental composition of the food waste throughout the year in relation to microbial population and biogas yield. More specifically, the study did not extend beyond 120 days of continuous operation to determine the long-term impacts of the continuous use of the method on the stability of the community. Also, the study did not examine on how the interaction between co-digestion with other organic waste streams that are prevalent in cities could affect the process.

6. Conclusion

Conclusively, this work on enhancing microbial community structure and its implication on biogas production from UFW in the United States of America has revealed the interaction between the digesters' operational variables, microbial interactions, and process efficiency. This study established that there is potential of increasing the biogas yields and the process stability if some of the important parameters like temperature, pH, organic loading rate, and mixing intensity are optimally controlled. The observed dynamics of microbial community and the fact that there are always multiple microorganisms capable of performing the same function prove that anaerobic digestion systems are well equipped to face the challenge posed by the variability of urban food waste. Accordingly, the study established that increases in temperature, slightly acidic pH and moderate OLR are optimum for the production of biogas albeit with risk of process instability. The concept of syntrophy and the significance of certain microbial factions at various phases of the digestion process were established, which paved way for specific enhancement approaches. The outcomes of the present study help to extend the existing literature on anaerobic digestion of FW from urban areas and provide useful recommendations for enhancing biogas generation in urban waste management facilities.

Recommendations for Future Research

- Undertake additional, large-scale, and comprehensible studies on the anaerobic digestion of UFW in order to confirm the results of this research in actual application. This should involve the long-term assessment of microbial population shifts, biogas yields, and system performance during the different seasons and in response to such factors as acclimatization over time (i.e., 1-2 years).
- Explore the possibility of using bioaugmentation strategies and the microbial consortia outlined in this study. This may include creating and evaluating specific inocula for early inoculation and high efficiency of anaerobic digesters treating municipal SW food waste. The research should concentrate on the enhancement of the composition as well as the method of application of these consortia for the best result.
- Examine the possibility of applying high-level pretreatment and the best microbial consortia. One such area could be to investigate how pretreatment methods such as physical, chemical or biological methods affect the adapted microbial consortia with a view of improving rates of hydrolysis and yield of biogas from the recalcitrant fractions in urban FW.
- Create and test models that describe the shifts in microbial communities, the characteristics of the substrate, and the operational conditions of the system. Such models should be designed to give real time optimisation advice regarding the anaerobic digestion systems dealing with urban food waste, given the fact that this waste is heterogeneous and its quality varies with time and season.
- Study the feasibility of integrating anaerobic digestion with other treatment processes of waste in the biorefinery concept. Such research could comprise, for instance, the potential linking of anaerobic digestion to composting, insect rearing, or hydrothermal carbonisation to get the highest yield from urban food waste.
- Carry out detailed life cycle assessments and techno-economic evaluations on optimized anaerobic digestions for cities' food waste stream. These kinds of studies should take into account such options as decentralized community scale digesters and large centralized plants in order to help in making the right policy measures and investments towards achieving sustainable urban waste management

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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