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Green waste addition to boosting solid-state anaerobic digestion of municipal solid waste for efficient methanogenesis and energy recovery

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Abstract

Biogas production from the organic fraction of municipal solid waste (OFMSW) is often hindered by the risk of acidic failure at high loadings due to its readily biodegradable nature. This study explored the potential of enhancing methane production through the co-digestion of OFMSW with green waste (GW) in anaerobic fermentation. By evaluating the impact of the carbon-to-nitrogen (C/N) ratio on digestion efficiency, we compared the performance of individual and co-digestion processes. The results demonstrated that co-digestion significantly outperformed individual digestion, achieving a maximum methane yield of 420 L/kgVS with an optimal OFMSW ratio of 2:1. At 10% total solids (TS) loading, this ratio led to a 72% improvement in mass and energy balance compared to the digestion of OFMSW or GW alone. Microbial community analysis revealed that *Clostridium* sp. was the dominant bacterium in the co-digestion process, while *Methanobacterium* sp. was the predominant archaea, both playing crucial roles in methane production. The co-digestion of the readily biodegradable OFMSW with the more slowly degradable GW proved to be an effective strategy for enhancing methane yield and energy recovery. These findings indicate the viability of co-digestion as a robust approach for optimizing methane production and advancing sustainable waste management and renewable energy generation.

Keywords: Organic fraction municipal solid waste, Green waste, Co-digestion, Methane production, Microbial community

1. Introduction

Municipal solid waste (MSW), driven by economic growth, urbanization, population increases, and enhanced lifestyles, presents a significant environmental dilemma globally. Urban MSW generation has reached alarming levels and projections indicate a continued upward trajectory [1]. Inadequate MSW management practices, including open dumping and burning, contribute substantially to environmental issues, such as greenhouse gas emissions, climate change, ocean pollution, and the depletion of natural resources. Life cycle assessment provides a methodological framework for evaluating the environmental impacts of a product or service throughout its lifespan, taking into account factors such as human toxicity, global warming potential, eutrophication, and mineral exploitation, as well as integrating economic assessments through the net present value to gauge the financial impacts [2, 3]. Recent data indicate that MSW is responsible for approximately 3% to 5% of global anthropogenic greenhouse gas emissions, exacerbating climate change and resource depletion [4]. Despite the potential for waste-to-energy conversion, which is not a novel concept, implementation in less economically developed countries remains limited. Thailand, in particular, possesses the capability to convert green market waste into renewable energy, yet it rarely utilizes it. This study aims to illustrate the environmental and economic benefits of converting green waste into renewable energy, advocating for sustainable waste management solutions [5, 6].

Environmental challenges have prompted the exploration of various waste management approaches, leading to a shift towards sustainable and eco-friendly technologies. Solid-state anaerobic digestion (SS-AD) has gained recognition as an effective method for waste treatment and energy recovery, providing an alternative to traditional composting by processing organic feed anaerobically to produce biogas and sludge [7]. Anaerobic digestion (AD) transforms organic matter into biogas, typically composed of 60–70% methane and 30–40% carbon dioxide [8]. The incorporation of co-digestion, which involves adding different organic materials to the digestion process, has proven to enhance SS-AD's efficiency. Research has demonstrated that incorporating green waste from households and green spaces into the anaerobic digestion process with municipal solid waste (MSW) enhances biogas production, methane generation, and overall energy recovery. It is worth mentioning that the organic part of MSW works better when mixed with other organics, e.g., yard waste (YW), food waste (FW), and pig slurry in anaerobic co-digestion (ACoD). Lowering YW content in the mix increases methane productivity, with a system using 20% YW feedstock achieving the highest methane output of 368.6 ± 21.6 mL/gVS after 45 days [9], indicating the potential of integrating green waste into SS-AD systems for improved energy recovery and waste management sustainability.

To contribute, this research explores the impact of integrating green waste into SS-AD processes for treating MSW. Specifically, it examines how green waste co-digestion enhances methanogenic activity and optimizes energy recovery, aiming to provide a deeper understanding of the dynamics and efficiencies of SS-AD when augmented with green waste, thereby offering a pathway to more sustainable waste management practices. By highlighting the potential benefits, such as increased methane production and improved energy yields, this research could significantly influence waste management policies and practices. The findings may be useful for developing waste treatment strategies that not only mitigate environmental impacts but also enhance renewable energy generation, contributing critical insights into the sustainability of waste management systems.

2. Materials and methods

2.1 Substrates and inoculum

MSW was collected from landfill sites in Nakhon Si Thammarat Province, Thailand, between December 16 and 25, 2016. Concurrently, Green Waste (GW), comprising grass clippings and fallen leaves, was gathered from the Faculty of Technology and Community Development at Thaksin University. Both types of waste were processed identically: air-dried at room temperature for 48 hours and then ground. The material was further refined through a 5-mm sieve to standardize the particle size, after which it was stored at 4°C to maintain its condition for subsequent experiments. Table 1 details the specific characteristics of the MSW and GW, illustrating their respective compositions and properties crucial for the research. For the anaerobic digestion experiments aimed at methane production, the inoculum was sourced from anaerobically digested sludge from the Pitak Palm Oil Factory’s biogas digester in Trang province. This sludge was enriched through gravity sedimentation and decanting to remove the supernatant, resulting in a volatile suspended solids (VSS) concentration of 20.7 g/L, which is ideal for fostering a robust microbial population necessary for effective methane generation [10].

2.2 Anaerobic co-digestion of MSW with GW

For the biogas production evaluation, the study designed experimental conditions varying both the total solids concentrations and the ratios of MSW to GW. Concentrations of 10%, 20%, 30%, 40%, and 50% total solids were tested alongside MSW: GW ratios of 1:1 and 2:1 to examine their impact on the anaerobic digestion process. These variations were assessed using the Biochemical Methane Potential (BMP) test. To establish anaerobic conditions necessary for the digestion process, the inoculums and substrates were mixed thoroughly using a hand mixer and then flushed with nitrogen gas. The mixtures were then placed into 0.5 L serum bottles with a working volume of 0.3 L, sealed with butyl stoppers to maintain anaerobic conditions, and incubated in a temperature-controlled environment at 35°C. Throughout the 45-day testing period, duplicate bottles were used for each set of conditions to ensure the reliability and reproducibility of the results. A control group, consisting of inoculum mixed with water, was also included to provide a baseline for comparative analysis.

The performance of each experimental setup was monitored daily using the displacement method to measure biogas production, and the composition of the biogas was analyzed through gas chromatography. In addition to these measurements, the microbial communities driving the anaerobic digestion were investigated using polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE). This microbial analysis offered insights into the diversity and dynamics of the populations involved, further enhancing the understanding of how different solids concentrations and substrate ratios affect biogas production. By integrating BMP tests, daily gas monitoring, and advanced PCR-DGGE techniques, the study aimed to comprehensively assess the influences of varying experimental conditions on the efficiency of biogas production. The findings are expected to inform strategies to optimize anaerobic digestion processes, contributing to the development of more efficient and sustainable waste management practices and renewable energy solutions.

2.3 Microbial community analysis

Nested Polymerase Chain Reaction-Denaturing Gradient Gel Electrophoresis (Nested PCR-DGGE) was utilized to elucidate the microbial community structure in the anaerobic digestion system during the co-digestion of MSW with GW, as per the methodology outlined by [11]. Genomic deoxyribonucleic acid (DNA) was extracted from sludge samples using QIAamp DNA mini kits from QIAGEN®. This genomic DNA then served as the template for subsequent polymerase chain reaction (PCR) amplifications targeting specific regions of the archaeal and bacterial 16S ribosomal (rRNA) genes. For archaea, the primer pairs Arch21f and Arch958r were initially used, followed by a second amplification with 340f-GC and 519r. Similarly, for bacteria, the initial amplification was performed using the primer pairs 27f and 1492r, followed by a secondary amplification with 357f-GC and 518r. These steps enabled the detailed analysis of the microbial community composition.

The denaturing gradient gel electrophoresis (DGGE) analysis was conducted using an 8% polyacrylamide gel in a D-Code system from Bio-Rad Laboratories, with a denaturant gradient of 40% to 60%. This technique allowed for the separation of DNA fragments based on sequence variations, providing a visual representation of the microbial diversity. Selected bands from the DGGE gel were subsequently excised, re-amplified to enrich the DNA, and purified. The enhanced PCR products were then sent to Macrogen Inc. (Seoul, Korea) for sequencing. The resulting partial 16S rRNA sequences were analyzed against The National Center for Biotechnology Information (NCBI) database using the nucleotide-BLAST (blastn) platform to identify the closest microbial relatives, offering crucial insights into the composition of the microbial community within the anaerobic digestion system.

2.4 Analytical methods

The chemical and physical properties of components involved in the anaerobic co-digestion process, including MSW, GW, inoculums, and digestate, were analyzed using standard methods as detailed in Standard Methods for the Examination of Water and Wastewater [12]. Total Solids (TS) and Volatile Solids (VS) assessments quantified the organic and inorganic contents of the samples, shedding light on their biodegradable fractions. The pH levels were measured to determine the acidity or alkalinity, which influences microbial activity essential for effective anaerobic digestion. Total Kjeldahl Nitrogen (TKN) was determined to assess the nitrogen content crucial for microbial growth and biogas production. Moreover, Total Volatile Fatty Acid (VFA) levels were measured to monitor the progression of the digestion process, while alkalinity testing helped evaluate the samples' buffering capacity, maintaining stable pH conditions conducive to microbial activity. The composition of lignin, cellulose, and hemicellulose, key structural components affecting biodegradability, was determined using methods suggested by [13].

Biogas production was thoroughly recorded daily using the water displacement method [14], and its composition analyzed via gas chromatography equipped with thermal conductivity detectors (TCD). Methane, carbon dioxide, hydrogen, and nitrogen levels were specifically measured under defined conditions, including the use of a 3.3 ft stainless steel column packed with Shin Carbon (60/80 mesh) and argon as the carrier gas at a 14 ml/min flow rate. Samples were injected in duplicate to enhance measurement accuracy [10]. Theoretical methane potential was calculated using Bushwell's formula, considering the stoichiometric conversion of compounds to CH_4, CO_2, and NH_3. The volatile solids in MSW and GW were presumed to primarily consist of carbohydrates ($C_6H_{10}O_5$) [15]. Methane and energy yields were quantified in m^3 CH_4 per ton of wet weight, and the thermal energy content of methane was estimated using its lower calorific value of 50.1 MJ/kg-CH_4 [15], providing critical insights into the energy potential of the biogas produced during the anaerobic co-digestion.

2.5 Kinetic model analysis

The estimation of methane yield, methane production rate, and lag phase time in the SS-AD process was carried out using the modified Gompertz equation (1).

$$Y(t) = Y_{max} \times \exp\left[-\exp\left(\frac{R_{max} \times e}{Y_{max}} \times (\lambda - t) + 1\right)\right] \quad (1)$$

Y represents the cumulative methane yield based on VS added (mL CH_4/g-VS) at a certain digestion time t (days). Y_{max} signifies the maximum methane yield based on VS added (mL CH_4/g-VS) achieved at the end of the digestion process. R_{max} denotes the methane production rate (mL CH_4/g-VS·d), while λ represents the lag phase time (day). The mathematical constant 'e' is equal to 2.7183. The parameters Y_{max}, R_{max}, and λ were estimated by a nonlinear curve fit in SigmaPlot 11.0 [16]. This mathematical modeling allowed us to gain insights into the kinetics of methane production during the SS-AD process. The hydrolysis constants (kh) were determined using the first-order kinetic model in equation (2).

$$\ln = \frac{B_0}{B_0 - B_t} k_h t \quad (2)$$

B_t is the cumulative methane yield (mL CH_4/g-VS) at the time (t), and B_0 represents the ultimate methane yield of the substrate. The hydrolysis constants (kh) provided essential information about substrate degradation rates during anaerobic digestion. To assess the energy potential of the produced biogas, the methane content was utilized to determine the energy content. The total energy potential of the biogas was calculated by considering the total volume of biogas produced during the anaerobic digestion process. This estimation allowed for the evaluation of the energy recovery potential from the biogas generated during SS-AD. The energy conversion efficiency of the SS-AD system was assessed by comparing the energy output (biogas energy content) with the energy input (initial energy content of the feedstock).

3. Results and discussion

3.1 Compositions of municipal solid waste and green waste

Table 1 delineates the characteristics of MSW and GW, shedding light on their composition which is integral for evaluating their suitability for anaerobic digestion and subsequent biogas production. MSW was found to have a TS content of 26.4%, contrasting with GW's higher TS content of 37.6%. The VS content, indicative of organic material conducive to biogas production, measured 18.3% in MSW and 30.3% in GW, showcasing both waste types as viable substrates. Nevertheless, the pH values of MSW and GW were recorded at 5.6 and 5.1, respectively, which are below the optimal anaerobic digestion range. This necessitates the adjustment of pH levels to between 7.0 and 7.5 through alkaline addition to foster a conducive environment for microbial processes.

Regarding moisture content, crucial for effective microbial activity, MSW registered 73.6% and GW 63.3%. These levels disclose the importance of maintaining adequate moisture for efficient digestion and biogas generation. Ash content, representing the inorganic fraction at 8.0% in MSW and 7.2% in GW, does not contribute to biogas production but indicates the presence of non-biodegradable material. The lipid and protein contents—vital substrates for anaerobic microbes—were 2.9% and 3.3% in MSW, and 1.3% and 1.7% in GW, respectively. MSW also exhibited a significant carbohydrate content of 65.3% of VS, compared to GW's 23.3%, highlighting MSW's superior biogas potential. Additionally, the fibrous materials like cellulose and hemicellulose showed higher percentages in GW (36.3% and 35.6% respectively), compared to MSW (8.4% and 7.3% respectively), suggesting GW's potential as a beneficial co-substrate. Lastly, lignin content was notably higher in GW at 20.6% compared to 3.3% in MSW, impacting the overall degradation process.

Lignin content significantly impacts the biodegradability and biogas potential of waste streams, with the carbon (C), hydrogen (H), oxygen (O), and nitrogen (N) contents playing crucial roles in their anaerobic digestion profiles. Analysis reveals that MSW contains a higher carbon content of 51.1% compared to GW 49.1%, with only minor differences in H, O, and N levels between the two streams. This chemical characterization underscores the suitability of both MSW and GW as substrates for anaerobic digestion and biogas production. Specifically, MSW's higher carbohydrate content suggests it has a greater intrinsic potential for biogas production. Nonetheless, GW, with its elevated levels of cellulose and hemicellulose, offers considerable advantages as a co-substrate, potentially enhancing the efficiency of biogas production during co-digestion processes. These insights are critical for optimizing anaerobic digestion strategies, aiming for sustainable waste management and renewable energy generation.

The specific composition of MSW used in this study, sourced from a landfill in Nakhon Si Thammarat, Thailand, primarily consisted of food, green, fruit, and vegetable waste, revealing a TS content of 26.36% and a VS content of 18.34%, as detailed in Table 1 [17]. These organic components, particularly lipids and proteins, are valuable substrates that significantly contribute to biogas production [18]. Comparatively, GW possesses a higher TS and VS content, indicative of a richer organic matter presence crucial for more efficient biogas generation. This suggests that blending MSW with GW could substantially boost MSW's biogas yield due to the higher biodegradable content in GW. Furthermore, the lower moisture content in GW compared to MSW might lead to enhanced process stability during co-digestion. Achieving an optimal moisture balance is essential for maintaining favorable conditions for microbial activity, with co-digestion serving as a strategic approach to optimize such conditions. The projected theoretical methane yield for high-moisture MSW stands at 576 mL/g-VS [17], highlighting the potential of integrated waste management strategies to improve the efficacy and sustainability of biogas production systems.

The comparative analysis of MSW and GW highlights their distinct compositions and the implications for biogas production. MSW, with its higher carbohydrate content, inherently possesses a greater potential for biogas production compared to GW. This advantage is further bolstered by MSW's richer lipid and protein content, which are critical organic sources that contribute significantly to biogas generation. Conversely, GW, although lower in these specific organics, is rich in cellulose and hemicellulose, making it an excellent co-substrate. These fibrous materials enhance the biogas production potential when co-digested with MSW by improving the overall breakdown and conversion of organic materials. Additionally, GW's higher lignin content, which typically hinders the degradation process during anaerobic digestion, can be effectively managed through co-digestion with MSW. This strategy helps in breaking down the lignin-rich fibers in GW, thereby optimizing the digestibility and gas yield.

However, the process of anaerobic digestion is not without challenges. The rapid production of volatile fatty acids (VFAs) from carbohydrates and free ammonia from proteins in MSW can act as potential inhibitors, affecting the stability and efficiency of the digestion process, which might impede biogas production [19]. These inhibitors necessitate careful management to maintain process stability. Despite these challenges, the high organic content in MSW and the complementary properties of GW underscore their utility as substrates for anaerobic digestion. While the low carbon-to-nitrogen (C/N) ratios of both waste streams are not ideal, they still provide a conducive environment for efficient digestion. Addressing the potential inhibitors in MSW during mono-digestion is crucial for optimizing the anaerobic digestion process, ensuring effective biogas production and advancing sustainable waste management practices.

Table 1 Characteristics of municipal solid waste (MSW) and green waste (GW).

Parameter	MSW	GW
TS (%)	26.4	37.6
VS (%)	18.3	30.3
pH	5.6	5.1
Moisture (%)	73.6	63.3
Ash (%)	8.0	7.2
Lipid (% VS)	2.9	1.3
Protein (% VS)	3.3	1.7
Carbohydrate (% VS)	65.3	23.3
Cellulose (% VS)	8.4	36.3
Hemicellulose (% VS)	7.3	35.6
Lignin (%VS)	3.3	20.6
C (%)	51.1	49.1
H (%)	6.6	6.4
O (%)	40.3	43.4
N (%)	2.0	1.1

3.2. Biogas production from co-digestion of municipal solid waste and green waste

The efficiency of the anaerobic co-digestion process utilizing MSW and GW was systematically evaluated by varying the MSW to GW ratios (1:1 and 2:1) and total solids (TS) concentrations (10%, 20%, 30%, 40%, and 50%). Key performance metrics such as methane yield, methane production rate, VS removal efficiency, and the hydrolysis constant were measured. Moreover, the data were analyzed using the Modified Gompertz model to fit the experimental outcomes. At a 1:1 ratio of MSW to GW, methane yield was observed to vary significantly, ranging from 14.73 to 311.03 mL-CH_4/g-VS, with the peak yield occurring at the lowest TS concentration of 10%. An increase in TS concentration consistently led to a decrease in methane yield, bottoming out at 50% TS. This trend was mirrored in the methane production rates, which spanned from 0.33 to 6.91 mL-CH_4/g-VS/day, achieving the highest rate at 10% TS. Similarly, the efficiency in VS removal declined as TS concentrations increased, showing a drop from 69.12% at

10% TS to 3.27% at 50% TS. The hydrolysis constant, indicative of the substrate degradation rate, also peaked at 10% TS, demonstrating a direct correlation with both methane yield and production rate.

Exploring a higher MSW to GW ratio of 2:1 revealed similar patterns. The methane yields here ranged from 26.52 to 420.54 mL-CH_4/g-VS, with the maximum again recorded at 10% TS. As with the 1:1 ratio, higher TS concentrations led to diminishing methane yields, reaching the lowest yield at 50% TS. Methane production rates at this ratio varied from 0.59 to 9.35 mL-CH_4/g-VS/day, with the optimal rate occurring at 10% TS. The VS removal efficiency exhibited a similar trend; it was highest at 10% TS (93.45%) and decreased significantly at 50% TS (5.89%). The hydrolysis constant values, reflecting the substrate degradation rate, again showed a peak performance at the lowest TS concentration, indicating that lower TS concentrations generally enhance the efficiency of methane production and substrate degradation in anaerobic co-digestion processes. These results (Figure 1A, 1B) highlight the critical influence of TS concentration on the efficiency of biogas production from co-digested waste streams.

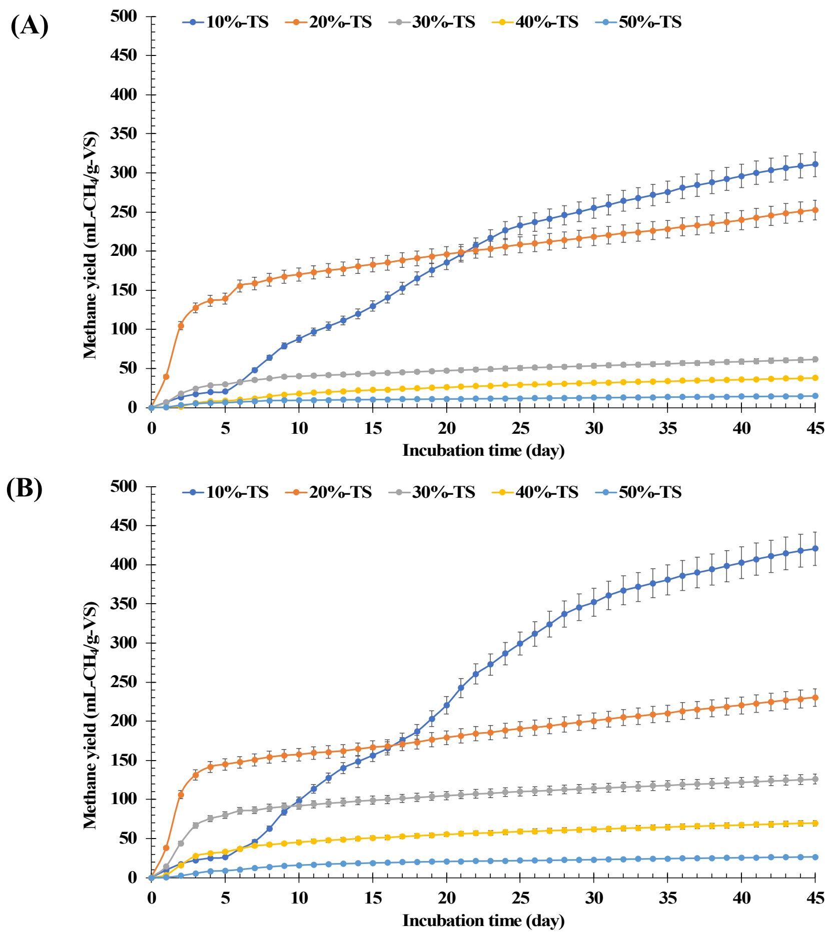

Figure 1 Accumulative methane yield of municipal solid waste (MSW) and green waste (GW); (A) = MSW: GW (1:1) w/w, (B) = MSW: GW (2:1) w/w ratio by anaerobic co-digestion process.

The Modified Gompertz model was used to fit the experimental data, providing insight into the kinetics of the anaerobic co-digestion process. The 1:1 MSW to GW ratio model fits well with methane yield and production rate at all TS concentrations. However, the lag time could not be determined (ND) due to the model's inability to fit the data adequately. At the 2:1 MSW to GW ratio, the model fit well for methane yield and production rate at all TS concentrations, and the lag time was determined. The results indicate that co-digestion of MSW and GW can enhance the biogas production efficiency compared to the mono-digestion of either waste stream. The highest methane yield and production rate were observed at the 10% TS concentration for both MSW to GW ratios, indicating that this TS concentration is optimal for maximizing biogas production. The VS removal efficiency decreased with increasing TS concentration, suggesting that higher TS concentrations may hinder digestion. The results demonstrate the potential of anaerobic co-digestion of MSW and GW for efficient biogas production.

The results showed that the MSW/GW ratio of 2/1 exhibited the highest methane yield, followed by the ratio of 1/1. Over the 45-day test period, the MSW/GW ratio of 2/1 achieved the highest methane yield of approximately 420.54 mL CH_4/g-VS, while the ratio of 1/1 produced around 311.03 mL CH_4/g-VS (Table 2). Since GW was the main substrate and consisted of lignocellulosic materials, it took some time for biogas production to initiate. Additionally, the lower MSW/GW ratio (1/1) resulted in a higher GW concentration than the MSW/GW ratio of 2/1. This inverse relationship between MSW/GW ratio and methane yield might be attributed to lower methanogenic activity and fewer methanogens in the digesters, leading to the accumulation of VFA produced during the acidogenic step. High concentrations of VFAs could potentially inhibit the methanogenesis process [20]. This observation suggests that MSW is readily biodegradable, while GW, due to the presence of lignin (41.61%), is more recalcitrant to biological degradation. The BMP process was accomplished for the tests with the MSW/GW ratio of 2/1, achieving the highest biodegradation (based on VS) of 93.45%. This high biodegradation indicates that the lignocellulosic components in GW were effectively degraded to produce biogas. It is well-known that lignocellulose, due to its high carbon-to-nitrogen (C/N) ratio, degrades slowly, leading to slow biogas production [21]. The BMP test demonstrated that the co-digestion of MSW and GW can be a practical approach for biogas production. The MSW/GW ratio of 2/1 showed the highest methane yield, while the ratio of 1/1 also exhibited considerable methane production. The results suggest that MSW, being more biodegradable, contributes to faster biogas production while GW, due to its lignocellulosic nature, requires more time to degrade biologically. The successful biodegradation achieved with the MSW/GW ratio of 2/1 indicates the potential of this co-digestion strategy for efficient biogas production from the lignocellulosic waste stream.

The TS loading plays a crucial role in anaerobic digestion, as it can influence the hydrolysis process, VFA production, and overall process stability of SS-AD reactors. Rapid hydrolysis can occur at excessively high TS loading, leading to the overproduction of VFAs. This can adversely affect the process stability of SS-AD reactors. Conversely, low TS loading may delay the start-up of digestion and impact the overall performance of the anaerobic digestion process. Hence, optimal TS loading is essential to achieve efficient biogas production. The present study found the optimal condition for biogas production from co-digestion of MSW and GW was at 10% TS content. Under this condition, methane yields ranged from 311.03 to 420.54 mL CH_4/g-VS, as shown in Table 2. However, as the TS content increased to 50%, the methane yields decreased to 26.52-69.8 mL CH_4/g-VS, representing a 94-95% reduction compared to the optimal condition. These results suggest that higher TS content negatively impacted the overall methane yield, and the best performance was achieved at lower TS loading.

Table 2 The efficiency of the anaerobic co-digestion process with municipal solid waste (MSW) and green waste (GW).

MSW:GW (1:1 w/w)	Methane yield (ml-CH_4/g-VS)	Methane production (ml-CH_4/g-feedstock)	VS removal (%)	Hydrolysis constant (d^{-1})	Digestion times (day)[a]	Methane production rate (ml-CH_4/g-VS/day)	Lag time (day)
10%-TS	311.03	65.6	69.12	0.087	40	11.24	3.14
20%-TS	252.65	49.5	56.14	0.068	39	10.37	ND.
30%-TS	61.53	15.3	13.67	0.073	42	2.56	ND.
40%-TS	37.95	10.2	8.43	0.078	41	1.37	ND.
50%-TS	14.73	2.3	3.27	0.076	44	0.62	ND.
MSW:GW (2:1 w/w)							
10%-TS	420.54	72.3	93.45	0.091	41	15.00	4.47
20%-TS	230.18	43.2	51.15	0.069	40	8.73	ND.
30%-TS	126.02	36.8	28.01	0.078	36	9.46	ND.
40%-TS	69.80	18.2	15.51	0.081	42	3.29	ND.
50%-TS	26.52	9.5	5.89	0.082	42	1.31	ND.

a=90% of accumulative methane production

The BMP tests conducted on MSW and GW at higher TS content of 50% for both 1:1 and 2:1 ratios revealed notably low biodegradation rates, ranging from 3.27% to 5.89%. This diminished biodegradation can be attributed to several factors that restrict microbial efficiency, including reduced mass transfer and lower diffusion coefficients associated with higher TS content. Furthermore, the lower water content at these higher TS levels likely exacerbated the inhibition of microbial activity, leading to a subsequent decrease in methane production. This observation aligns with findings from other studies involving different feedstocks, such as yard trimmings and corn stover, which reported significant reductions in total methane yield—by approximately 25% to 38%—when TS content was increased from 22% to 30% during anaerobic digestion processes. Consequently, the most effective TS loading for the co-digestion of MSW and GW was identified at 10%, a level that facilitated the highest methane yields. Higher TS concentrations

(40-50%) were found to further impede methane production, likely due to the combined effects of low water content and decreased mass transfer, which hinder overall microbial activity.

The microbial community composition in the AD reactors, crucial for the co-digestion of MSW and GW, was delineated using DGGE. This analysis highlighted significant bacterial and archaeal entities integral to the process (Figure 3A, 3B). The bacterial spectrum was dominated by genera such as *Clostridium*, *Acetomicrobium*, *Bacteroides*, *Gramella*, and *Desulfovibrio*. *Clostridium* is particularly notable for its role in unintentional oxygen utilization during acidogenesis and its production of hydrolytic enzymes, contributing significantly to syntrophic acetate oxidation [22]. *Acetomicrobium*, on the other hand, is a bacterium that ferments starch, glycerol, monosaccharides, and organic acids [23]. *Clostridium* and *Acetomicrobium* contribute to acetic acid production via fermentation [24]. *Bacteroides*, another key bacterial genus in the AD flora, can hydrolyze lignocellulosic biomass. Previous studies have shown that augmenting *Bacteroides* and *Clostridium*-rich methanogenic consortium in high-solid AD led to an increase in *Bacteroidales* and *Clostridiales*, resulting in a methane yield of 376.7 mL-CH_4/g-VS [22]. *Gramella*, prominent in our previous study on the two-stage fermentation of municipal waste, is associated with the hydrolysis of polysaccharides such as laminarin, alginate, pachyman, and starch [25]. *Desulfovibrio*, a syntrophic propionate-oxidizing bacterium, and the Methanobacterium family, utilizes sulfate and lactate to generate acetate and hydrogen during AD processes [22].

In the archaeal community, the DGGE bands corresponded to *Methanobacterium, Methanoculleus, Methanomicrobium*, and *Methanobrevibacter* (Figure 3B). These archaeal genera are known as formate- or CO2-based hydrogenotrophic methanogens, and they were found to be prominent in our AD reactors. Similar archaeal genera have been commonly detected in AD processes involving food waste, municipal waste, cow manure, and other substrates [26]. The presence of abundant hydrogen-producing bacteria, as indicated by hydrogenase genes, was observed in our reactors. This aligns with findings from previous studies reporting the prevalence of hydrogen-producing bacteria such as *Clostridium, Acetomicrobium, Bacteroides*, *Gramella*, and *Desulfovibrio* [27-31]. Consequently, hydrogenotrophic methanogens, represented by *Methanobacterium, Methanoculleus*, *Methanomicrobium,* and *Methanobrevibacter,* were highly abundant in the AD process. Overall, the microbial community analysis provides valuable information about the key bacterial and archaeal species involved in the co-digestion of MSW and GW. Hydrogen-producing bacteria and hydrogenotrophic methanogens suggest a well-functioning AD system, contributing to efficient methane production. Understanding the microbial community dynamics in AD processes is crucial for optimizing and controlling biogas production, leading to more sustainable and efficient waste management strategies.

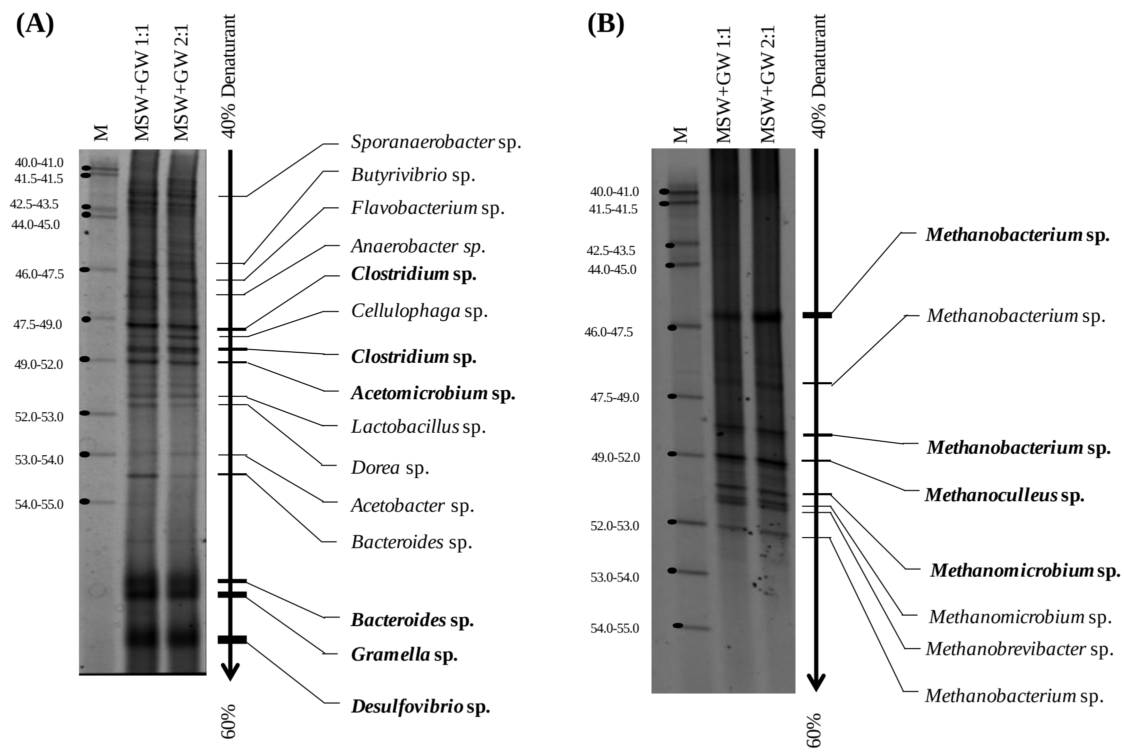

Figure 3 Bacterial (A) and archaeal (B) community in biogas production system from co-digestion of municipal solid waste (MSW) with green waste (GW) investigated by nested PCR-DGGE.

3.3 Energy production and mass balance

Energy production from organic waste, particularly MSW and GW, is a significant concern for sustainable waste management. In this study, we investigated the energy production potential of MSW and GW using a one-stage mesophilic anaerobic digestion process. For GW alone, the biogas production yielded an energy content of 4,212 MJ per ton. However, when co-digesting MSW and GW in the ratio of 1:1, the energy production significantly increased to 11,197 MJ per ton. Moreover, when the co-digestion ratio was adjusted to 2:1 of MSW and GW, the energy production further increased to 15,139 MJ per ton (Figure 4). This demonstrates that co-digestion of MSW and GW in the one-stage anaerobic digestion process leads to a substantial enhancement in energy generation compared to the digestion of GW alone. Specifically, when co-digesting MSW and GW at a total solid concentration of 10%, the one-stage anaerobic digestion process resulted in an impressive 72% increase in energy production for the 2:1 co-digestion ratio and a 62% increase for the 1:1 ratio when compared with the mono-digestion of GW. These findings highlight the advantages of co-digestion and the potential for harnessing more energy from organic waste through optimized waste ratios. Previous studies have explored the possibility of biohythane production using a two-stage anaerobic digestion process from MSW, reporting energy productions of 9,784 and 7,231 MJ per ton for two-stage and one-stage processes, respectively [17].

However, our study improved the C/N ratio to range from 16.88 to 33.79, which is considered an optimum condition for co-digestion of MSW and GW, resulting in higher energy recovery efficiency from organic waste. The mass balance of the one-stage anaerobic digestion process from the co-digestion of MSW and GW (Figure 4) demonstrates the successful conversion of organic waste into valuable biogas. Interestingly, when analyzing the co-digestion ratios of 2:1 and 1:1 at different total solid concentrations, it was observed that higher total solids content led to a lower biodegradation rate and an increase in the amount of organic matter remaining in the process, as evidenced by the remaining volatile solids. In conclusion, the co-digestion of MSW and GW using a one-stage anaerobic digestion process presents a promising approach for enhanced energy production from organic waste. The optimized co-digestion ratios and improved C/N ratio contribute to higher energy recovery efficiency, making this waste-to-energy process an environmentally friendly and economically viable option for organic waste management.

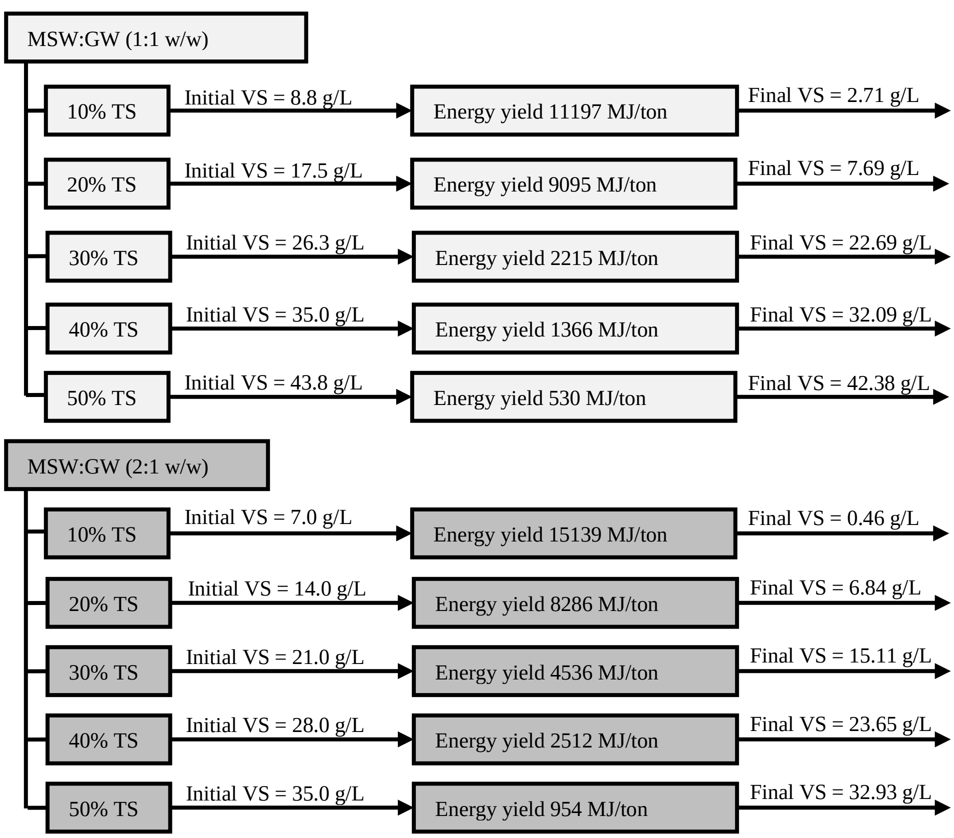

Figure 4 Energy recovery and mass balance from the municipal solid waste co-digestion and green waste anaerobic digestion process.

4. Conclusion

To sum up, the co-digestion of MSW and GW using the anaerobic digestion process has shown significant promise for enhancing biomethane production and energy recovery from organic waste. Of all the co-digestion ratios that were looked at, the 2:1 ratio of MSW and GW at a 10% TS concentration showed the most promise for making biomethane, with an impressive yield of 420 L/kgVS. It was shown that co-digestion could improve biodegradation and the C/N ratio of both MSW and GW, which would lead to more biogas production than GW alone's mono-digestion. The nitrogen-rich GW was especially helpful in changing the C/N ratio during the anaerobic digestion process, which further sped up the process of turning organic matter into biomethane. Overall, co-digestion presents a sustainable and efficient approach for converting organic waste into valuable biomethane, providing a viable solution for waste management and energy recovery. The success of this study shows how important it is to get the waste-to-carbon and carbon-to-nitrogen ratios just right in co-digestion processes so that biomethane production can reach its full potential. As a renewable and environmentally friendly energy source, biomethane derived from co-digestion holds great promise for contributing to a more sustainable and green future. Further research and implementation of co-digestion technologies could make a significant contribution to mitigating the environmental challenges posed by organic waste and meeting our society's growing energy demands.

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