



Research article

Enhancement of anaerobic digestion by co-digesting food waste and water hyacinth in improving treatment of organic waste and bio-methane recovery

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HIGHLIGHTS

- The effect of Co-digestion of FW and WH at different mix proportions was studied.
- Anaerobic Co-digesting WH & FW is stable compared to mono-digesting the substrates.
- Optimum mix of 70% WH & 30% FW produced 616.01ml/g-VS biogas & methane content of 71%.
- ANOVA showed a significant difference in biogas yields when WH was increased to 55% & 70%.
- The experimental findings were corroborated by the simulation using the modified Gompertz model.

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ABSTRACT

In Kenya, 57% of the municipal solid waste generated is Food waste (FW) which has high organic content. However, the treatment and bioconversion of FW to biogas have always been challenging due to its rapid biodegradation, resulting from rapid hydrolysis and accumulation of volatile fatty acids and lowering pH in the bioreactor. In this study, the anaerobic digestibility of FW as a mono substrate was compared to co-digestion of FW with water hyacinth (WH) for improved biogas production and organic matter removal efficiency in a laboratory batch reactor. Different mix proportions of FW and WH were co-digested under mesophilic conditions (37 °C) at a dilution of 6% (w/v) Total Solids (TS) content. The TS of the substrates (Food waste and Water Hyacinth) were pre-processed to have a concentration of TS at 6% (60 g/L) to operate a wet AD which requires the substrate to be less than 15% TS. The proportions of WH: FW (v/v) were 100:0, 85:15, 70:30, 55:45, 30:70, 15:85, and 0:100. In the batch reactors the anaerobic co-digestion was conducted with Substrate to Inoculum (S/I) ratio of 1:1. FW is generally considered to have high volatile solids which hydrolyze rapidly lowering pH arising from excess production of Hydrogen which in presence of CO₂ and acetogenic bacteria leads to more production of acetate, formate and other long chain fatty acids which inhibits methanogenesis as a result of rapid acidification. The rapid acidification of the bioreactors that are used to treat FW results in the inhibition of the methanogenesis process. The co-digestion of the substrates could have improved the process parameters by reducing acidity caused by the high C/N ratio, reducing the inhibitory range, and increasing the buffer capacity which enhanced the bio-methane potential and the microbial activity. The batch experiments were set in triplicate for both cases of FW, WH, mixtures, and Inoculum. The results showed that the average gas yields after 81 days for the various mix proportions were 256.27 and 357.69 ml/g-VS for mono-digestion of WH and FW respectively. For the mixtures of WH: FW the average reported biogas production were 305.01, 280.27, 548.91, 616.01 and 270.87 ml/g-VS for mixtures of 15:85, 30:70, 55:45, 70:30 and 85:15 respectively. The modified Gompertz model showed that the digesters with WH and FW alone had lag times of 2.599 and 1.052 days respectively. The mix substrates of WH: FW 85:15, 70:30, 55:45, 30:70 and 15:85 shown lag times of 2.456, 3.777, 2.574, 1.956 and 1.75 days respectively. A mix (WH: FW) of 70:30 had the highest maximum specific biogas production R_{max} and the maximum biogas production potential of 18.19 mlCH₄/gVS per day and

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607.7mlCH₄/gVS respectively. The R² and RSME values ranged from 0.9867 to 0.9963 and 2.663 to 9.359 respectively in all the digesters. The study shows that the co-digestion of WH and FW in the mix ratio of 70:30 improved the volume of biogas produced and organic matter removal efficiency reached 79%.

1. Introduction

Annually about 1.3 billion tons (one-third) of the world's food production is wasted in the food supply chain resulting in remarkable economic, social, and environmental problems (Xu et al., 2018). Food waste (FW) comprises a larger component of municipal solid waste which includes canteen and restaurant waste, food-processing waste, and household food waste. It is approximated that Europe and China lose approximately 100 million (Mega grams) Mg and 600million Mg respectively of food annually (Algapani et al., 2016). The FW trend is rapidly growing and it is projected that FW generation will go up by 44% and 51% in Europe and China respectively by the year 2025 (Algapani et al., 2016). According to Xu et al., 2018, the amount of FW generated in the United States has rapidly increased by 50% since 1974 and recent reports show that close to 38 million tons of FW are generated yearly with 76% of the generated FW being disposed of in the landfills. In Kenya, the problem posed by the management of food waste is real. There has been an increased rate of urbanization and rural-urban migration which has increased the volume of food waste generated within the urban centers. Food waste accounts for 57 percent of the total Municipal Solid Waste (MSW) generated in Kenya (Hesborn-Andole, 2016). It is estimated that the household solid waste generated in Kenya's capital City Nairobi per capita per day varies in volume from 0.4 to 0.7 kg/capita/day of which 57% of it is food waste (Kasozi A & Von, 2010). In Kenya, food waste comprises restaurant waste, household kitchen waste, institutional kitchen waste, and discarded expired food. According to the 2019 population census, the population of Kenya is approximated to be 47.6 million people (KNBS, 2019). 2019 population census indicates that about 28.5% of the total Kenyan population lives in urban areas translating to about 14.5 million people. Nairobi which is the capital city is estimated to have a population of 4.3 million people who generate approximately 2400 tons of solid waste daily of which 1368 tons is FW (57%) (Muiruri et al., 2020). Improper disposal of FW has resulted in the increase in the production of greenhouse gases in landfills resulting in global warming hence the need to explore suitable ways of managing and treating the FW (Algapani et al., 2016). When FW is dumped in the landfills, they undergo anaerobic degradation which results in the release of elements such as leachates and gases, concentrated Chemical Oxygen Demand (COD), volatile fatty acids (VFAs), methane, and ammonia which are harmful to the environment (Fisgativa et al., 2016). Due to this, landfills are considered the third-largest source of methane (CH₄) worldwide representing about 11% of the total emissions (Fisgativa et al., 2016).

Fisgativa et al. (2016) reported that biological treatment processes have been adopted as means for treating FW, which have high organic matter and water content that contributes to the harmful components in landfills. One of the biological treatment processes that have been proved to be effective in the treatment of FW is anaerobic digestion (AD) (Algapani et al., 2016). Due to the high volatile solids and moisture content, FW is generally considered to be a good substrate for the anaerobic digestion (Pagliacci et al., 2015). The biogas potential of feedstocks such as food waste is dependent on the concentrations of four major components: proteins, lipids, carbohydrates, and cellulose (Pagliacci et al., 2015). However, due to the high concentration of carbohydrates and low pH of FW, there is normally rapid acidification of the digesters that are used to treat FW which results in the inhibition of the methanogenesis process (Algapani et al., 2016). Anaerobic digestion is effective in the treatment of wastewater sewage sludge and animal waste however, its applicability for the treatment of FW has encountered numerous technical problems due to the inhibition of the processes as a result of the accumulation of volatile fatty acids causing process instability (Xu et al., 2018). Due to the inhibition effects, several technologies have

been used to improve the efficiency of the biodegradation of the complex FW. Ding et al., 2021 highlighted some of the countermeasures that have been suggested to solve the issue of reactor inhibition including two-stage anaerobic digestion and co-digestion. Naran et al., 2016 also indicated that enhancing the hydrolysis pretreatment needs to be carried out to improve the digestive efficiency of waste by solubilization of the decomposable organic substances which also reduces the time for anaerobic digestion. Liu et al., 2019 in his study suggested that the two-stage treatment and pretreatment processes were expensive and time-consuming hence leaving co-digestion as a viable option for anaerobic digestion of FW.

The water hyacinth (*Eichhornia crassipes*) is an invasive water plant that grows quickly. Its population can double in just five to fifteen days, and it can produce 140 million clone plants per year (Omondi et al., 2019). These aquatic plants have infiltrated water bodies, reducing dissolved oxygen and interfering with aquatic animal health (Brown et al., 2020). In East Africa, the plant has infiltrated and spread throughout Lake Victoria, causing economic and environmental issues such as obstructing water intakes, fishing areas, and transportation ways (Njogu et al., 2014). Water hyacinth has a high hollocellulose content and a low lignin content per unit volume, with 43 percent and 8–14 percent, respectively (Bhattacharya and Kumar, 2010). Hollocellulose refers to the cellulose and hemicellulose components of the substrate after the lignin has been removed. According to Li et al., 2021 lignin decreases the hydrolysis rate of lignin-wrapped hollocellulose and hence the bioconversion efficiency of AD, a phenomenon known as lignin inhibition. The lignin in water hyacinth was not removed from the substrate in the current investigation.

The fact that WH is hollocellulose in nature and has a low lignin content suggests that it could be used as a biomass crop in biogas generation (Lara-Serrano et al., 2016). Cellulose, hemicellulose, and lignin are linked in plant cell walls through a variety of covalent and non-covalent bonds to form a complex, compact structure (Li et al., 2021). Water hyacinth has a low lignin level of 7–26 percent and a high cellulose content of 18–31 percent and a hemicellulose content of 18–43 percent. In AD process, cellulose and hemicellulose components are easily broken down through the action of hydrolytic bacteria to produce sugars and short chain fatty acids which are converted to methane (Zhang et al., 2016). Unlike cellulose and hemicellulose, lignin cannot be degraded under anaerobic circumstances because the extracellular enzymes that breakdown lignin require oxygen to function (Fan et al., 2019). Lignin obstructs hydrolysis in two ways during the AD hydrolysis stage: by producing a protective barrier that blocks enzyme access and by reacting irreversibly/reversibly with enzymes due to its intrinsic hydrophobicity (Ju et al., 2013). As a result, water hyacinth contains approximately 40% carbohydrates, making it highly biodegradable (Brown et al., 2020). According to Bhattacharya and Kumar (2010), WH biomass is constituted of 33% hemicellulose and 20% cellulose. The basic components of lignocellulosic biomass, according to Paul and Dutta 2018, are cellulose (15–99 percent), hemicellulose (0–85 percent), and lignin (0–40 percent). WH also has a relatively high carbon-to-nitrogen ratio, which is a favorable feature in biomass for biogas synthesis (Omondi et al., 2019). Even though WH is low in lignin, its lignocellulose composition may be a disadvantage in biogas production by slowing the hydrolysis process and eventual conversion to biogas. The lignocellulose structure can restrict WH decomposition, resulting in sluggish digestion by bacteria and thus a loss in methane output (Li et al., 2013). Pretreatment, dilution, and co-digestion with another substrate are all approaches that can be utilized to improve the biodegradability of WH (Makofane et al., 2019). Among these approaches, co-digestion with highly biodegradable substrates is a simple and cost-effective strategy for enhancing WH biodegradation. FW is one such substrate because it is highly biodegradable and has a high organic content (Jingura et al., 2017).

Anaerobic digestion (AD) is a biological decomposition process that takes place in the absence of oxygen. It is carried out by various processes such as hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Wandera et al., 2018). In anaerobic digesters, the main operational conditions necessary for anaerobic digestion include pH, temperature, organic loading rate, and hydraulic retention time. These factors play a vital role in determining the reaction rates of the various processes (Matheri et al., 2017). However, the yield of biogas can be affected by the composition, origin, and content of the organic substance (Jingura and Kamusoko, 2017). The Bio-methane Potential (BMP), or biogas potential, which is the measure of the amount of biomethane that can be produced from various substrates varies depending on the chemical composition of the substrates. The chemical constituents that influence the BMP are proteins, carbohydrates, fats, and lignin. Jingura and Kamusoko (2017) in their review indicated that fats and protein produce more methane compared to carbohydrates whereas the biodegradability of lignin is low. The amount of organic matter in substrates is quantified by the COD, which indicates how much biogas can be produced from anaerobic digestion (Fisgativa et al., 2016). When the COD of a substrate is known, its theoretical methane potential can be calculated (Achinan and Euverink, 2016). However, high concentrations of soluble chemical oxygen demand may lead to the accumulation of Volatile Fatty Acids (VFA), slowing down the biogas production or lowering the pH of the reactors which has a great impact on the methanogenesis stage (Zahedi et al., 2018). The TS and VS are also important parameters in assessing the anaerobic digestion process efficiency, TS has an impact on the degradation rate of the substrates, bacteria growth rate, and the treatment efficiency of the reactor. The methane yield increases with the increase in the VS content and reduction in the hemicellulose. The BMP of the various substrates in the AD process is influenced by the concentrations of the VS (Garcia et al., 2019). The C/N ratio in a substrate represents the interrelationship between the amount of carbon and nitrogen present in biomass and it is an indicator of the nutrients available in the substrate. For the effective operationalization of the AD process, the C/N is one of the important parameters that need to be at the optimum level (Kothari et al., 2014). Apart from maintaining a suitable environment during the AD process, the C/N ratio also helps in controlling and maintaining proper nutrient balance for the development of the microorganisms. A depressed C/N ratio indicates that the substrate has a high protein content which results in an increase in the amount of free ammonia during the AD process resulting in high pH which causes the inhibition of methanogenesis (Algapani et al., 2016). On the contrary, a high C/N ratio causes a rapid reduction in nitrogen levels which contributes to low biogas production. Because of the accumulation of ammonia at low C/N ratios, the pH of the reactor can rise above 8.5, which in turn stifles the growth of methanogenic bacteria. When the C/N ratio is too high, however, there is not enough nitrogen to support gas generation (Wang et al., 2014). The C/N ratio may be brought into a more desirable range if the two substrates are co-digested. Lower gas yields occur when the C/N ratio is less than 25 percent due to the accumulation of ammonia when the PH rises over 8.5 due to nitrogen consumption by the methanogens (Matheri et al., 2017).

Although the anaerobic digestion of FW and WH has shown potential as a source of bio-methane there has not been a direct test to investigate the effect of co-digesting the FW and WH at different mix ratios under mesophilic conditions. This study determined the impact of co-digestion on; (i) Biogas production potential (ii) organic matter removal efficiency (iii) kinetics of substrate degradation using the Modified Gompertz model. The batch reactor was used to conduct the treatment of the organic waste and bio-methane recovery in an AD system.

2. Materials and methods

2.1. Inoculum & substrates

The inoculum used in this study was collected from an active anaerobic reactor that has been in operation for over one year in JKUAT. The

Table 1. Percentage of WH and FW, VS, and COD in different feeding substrates.

Substrate	WH (%)	FW (%)	VS g/l	COD (g/l)	COD/VS (gCOD/gVS)
D1	0	100	54.12	85	1.57
D2	15	85	48.5	65	1.23
D3	30	70	49.65	74	1.49
D4	55	45	50.23	72	1.43
D5	70	30	50.44	68	1.34
D6	85	15	46.43	78	1.67
D7	100	0	34.97	60	1.72

study involved the characterization of FW samples from the JKUAT staff cafeteria and WH growing along the open drains outside the gate of the JKUAT main campus. The two substrates were used as the feedstock in a batch study. The tests that were carried out on the substrates included elemental analysis, biochemical analysis, and physical analysis.

All the impurities such as bones, papers, and plastic bags from FW were removed. The FW was blended using a blender to obtain a slurry. The blended FW was kept in sterilized sampling bottles which were stored in the freezer at a temperature of 4 °C to preserve the sample. The stems of the WH were removed and the leaves were blended using a blender to obtain the slurry. The slurry was then packed in sterilized sampling bottles and stored in the freezer at a temperature of 4 °C. Before feeding, the substrates were diluted with water to obtain a TS content of 6% (w/v) wet weight. The inoculum/substrate ratio was 1:1 to ensure enough microorganisms at the beginning of the batch process.

2.2. Batch experiments setup and operation

The batch experiments were set up in triplicates by use of 120 ml serum bottles with a working volume of 80 ml. The substrates were prepared at different mix ratios of WH to FW (v/v) as shown in Table 1. The mix ratios of WH and FW had VS and COD concentrations in the range of 34.97–54.12 and 68–85 g/l respectively. In the serum bottles, 40 ml of inoculum was put with the remaining 40 ml being filled with the substrate. After adding the designed amount of inoculum and the substrates in the serum bottles, the headspace of each of the serum bottles was purged with nitrogen gas (99% purity) for about 2 min to ensure that anaerobic conditions were obtained. The serum bottles were then closed tightly by use of rubber cocks and then sealed by use of sealing tapes. The serum bottles were then placed inside an incubator that was maintained at a mesophilic temperature of 37 °C.

Nam et al., 2016 in their study on Enhancing biogas production by anaerobic co-digestion of water hyacinth and pig manure used the ratios of 0:100, 20:80, 40:60, 50:50, 60:40, 80:20, and 100:0. Further Haider et al., 2015 in his investigation of Effect of mixing ratio of food waste and rice husk co-digestion and substrate to inoculum ratio on biogas production applied co-digestion ratios of FW: RH of 91:9, 55:45, 30:70 and 15:85. Another study conducted by Emmanuel Pax et al. (2020) used the ratios of 50:50, 67:33, 33:67 and 25:75. Hence based on these findings this research adopted the use of intervals of 15% since this was close to the various types of research that were conducted using similar substrates to the ones used in this research.

2.3. Kinetic analysis of gas production potential

From the previous studies the results have revealed that when the substrate is a complex organic matter, the gas cumulative curves tend to appear as gentle curves which are characterized by maximum gas production rate, lag phase period, and maximum cumulative gas production potential parameters. These parameters are estimated by the use of the modified Gompertz model (Wandera et al., 2018). Using the model eq. (1), a nonlinear regression analysis was performed using MATLAB R2021b software to determine the kinetic parameters of Rmax, G0, and λ. To validate the accuracy of the model, the coefficient of determination (R²) and root mean square error (RMSE) were computed for the model. The

coefficient of determination shows the goodness of fit index which was determined by MATLAB R2021b software. Standard deviation between the predicted and experimental values were inferred from RMSE, eq. (2) with a lower value of RMSE indicating a better fit (Pramanik et al., 2019).

$$G(t) = G_0 \times \exp \left\{ - \exp \left[\frac{R_{max} \times e}{G_0} (\lambda - t) + 1 \right] \right\} \quad (1)$$

where G (t) is cumulative methane yield at time t (mLg^{-1} VS).

G_0 Is the methane potential of the feedstock (mLg^{-1} VS).

R_{max} Is the maximum methane production rate (mLg^{-1} VS).

λ is the phase period in hours.

t is incubation time in hours.

e = 2.718282.

$$RSME = \sqrt{\frac{\sum_{i=1}^n (PV_i - MV_i)^2}{n}} \quad (2)$$

where PV_i is the predicted value of biogas volume, MV_i is the experimental value of the biogas volume, and n is the number of measurements.

2.4. Bioenergy conversion potential of the substrates

Based on the elemental composition of the substrates, the theoretical bio-methane potential (TBMP) of FW & WH was stoichiometrically calculated by using Boyle's equation (Aragon et al., 2017) where the constants a, b, c, and d represent the molar fraction of C, H, O, and N, respectively. The elemental composition of the substrates was established from the elemental analysis of the substrate. The assumption in Boyle's equation was based on the complete stoichiometric conversion of a substrate with no distinction between biodegradable and non-biodegradable fractions (Nielfa et al., 2015; Raposo et al., 2012).

$$TBMP(mLCH_4gVS^{-1}) = \frac{22.4 \times \left(\frac{a}{2} + \frac{b}{8} - \frac{c}{4} - \frac{3d}{8} \right)}{12.017a + 1.0079b + 15.999c + 14.006d} \quad (3)$$

2.5. Gas quality analysis

The determination of gas quality was done by the use of the gas chromatograph method. Syringes were fitted with a self-sealing rubber septum for gas sampling. 1 ml gas samples from the headspace gas were taken using an airtight syringe and injected into gas chromatographs (Models GC-8A a, Shimadzu Corp., Kyoto, Japan for Gas Analysis gas quality). The gas chromatograph for gas quality determination was fitted with a thermal conductivity detector and a Poropak N column and standard gas was injected for comparison with the sample using retention times. The initial and final temperatures of the column were maintained at 1200 °C with the injecting temperature being at 1500 °C. The run time for the test was 7 min at a column length of 3 m. The mobile phase was Helium Gas with 99.9% purity.

2.6. The organic matter removal efficiency

In determining the organic matter removal efficiency, Total Solids (TS), Volatile Solids (VS), and Chemical Oxygen Demand (COD) were determined at the beginning and the end of the batch experiment under different mix proportions. The removal efficiency was calculated and the results as indicated in Table 3. The removal efficiency was calculated as per Eq. (4) (Hallaji et al., 2019).

$$\left(\frac{A - B}{A} \right) \times 100 \quad (4)$$

where A is the initial value of TS, VS, or COD at the beginning of the batch experiment and B is the final value of TS, VS, or COD at the end of the batch experiment.

2.7. Analytical methods

Analysis of the Total Chemical Oxygen Demand (TCOD), Chemical Oxygen Demand (COD), ammonia, PH Total Solids (TS), and Volatile Solids (VS) were conducted following the methods outlined in the standard methods (APHA 2005). The TS & VS were determined by drying the sample in an oven at temperatures of 103 °C–105 °C and consequently igniting it in a muffle furnace at about 550 °C for 1 h and thereafter the sample was cooled in a desiccator and then weighed. The igniting was repeated until the weight change was less than 4%. The analysis was done in triplicates and the TS & VS were calculated.

The dichromate method was used to determine chemical oxygen demand (APHA 2005). About 2.5 ml of the sample was placed in digestion tubes thereafter a digestion solution of acidified potassium dichromate was added, and concentrated sulphuric acid was run down inside the tubes. Soluble chemical Oxygen (SCOD) was run on samples filtered through 0.45 mm filters. The digestion samples were then placed inside a COD reactor at 150 °C for 2 h after which the samples were cooled at tested by use of a spectrometer. The biogas production was measured by the use of syringes and the measurements were done daily.

Methane content was analyzed by gas chromatographs (Models GC-8A a Shimadzu Corp., Kyoto, Japan for Gas Analysis gas quality). The elemental analysis was done by use of the Atomic Absorption Spectrometer (AAS) iCE 3300. Triplicates were used for the analysis of the various properties of the substrates. The modified Gompertz equation was used to fit the data of cumulative methane in MATLAB R2021b to predict the methane production (P).

2.8. Statistical analysis

One-way Analysis of variance (ANOVA) was used to evaluate significant differences in cumulative biogas production, with p-values less than 0.05 considered to be significant. For repeated comparisons of variables based on varied digestion durations mix proportions of the experiment, Tukey's honestly significant difference (HSD) post-hoc tests were used. All of the numbers in the tables and graphs are averages with standard deviations (SE). Data were checked and transformed as appropriate to meet the normality and variance homogeneity requirements before statistical analysis. The analysis was performed by using the statistical software IBM SPSS 20.0.

3. Results and discussion

3.1. Characterization of the substrates

The physiochemical characteristics of WH and FW are indicated in Table 2.

Table 2. Characteristics of food waste, water hyacinth, and inoculum.

Parameter	Unit	FW (n = 3)	WH (n = 3)	Inoculum(n = 3)
TS	g/L	60.78 ± 0.73	62.20 ± 0.29	71.48 ± 0.63
VS	g/L	54.12 ± 0.97	34.97 ± 0.38	45.09 ± 5.41
VS/TS	/	89.05	71.83	63.08
TCOD	g/L	170 ± 2.06	140 ± 1.24	80.23 ± 1.55
SCOD	g/L	85 ± 2.32	60 ± 1.69	32.41 ± 0.28
pH	/	5.8 ± 0.34	6.4 ± 0.16	7.73 ± 0.11
NH ₄ -N	g/L	0.97 ± 0.05	4.98 ± 0.17	3.09 ± 0.37
C	% (DM)	45.97 ± 0.48	21.57 ± 0.59	-
O	% (DM)	18.56 ± 0.92	28.28 ± 0.70	-
N	% (DM)	2.66 ± 0.24	2.049 ± 0.06	-
H	% (DM)	16.44 ± 0.69	5.19 ± 0.36	-
C/N Ratio	% (DM)	17.28 ± 0.57	10.53 ± 1.01	-

Notes: DM means dry matter.

3.2. C/N ratio

Based on the elemental analysis WH was noted to have less carbon content than FW. The observation indicates a lesser potential for biogas production for WH compared to FW. The (C/N) ratios of WH and FW were 10.53 and 17.28 respectively which fell within the recommended range of 8–20 for ideal biogas generation as reported by (Omondi et al., 2019). Wang et al., 2012) in their study also reported values of 9–30 as suitable for anaerobic digestion. The substrates with a higher C/N ratio provide more carbon for the production of methane. The C/N ratio is an important parameter in the anaerobic digestion process of the substrates since it impacts the concentration of Total Ammonia Nitrogen (TAN) and Free Ammonia Nitrogen (FAN) which causes an inhibitory effect on the methanogens during the methane production stage (Paul et al., 2018). Ammonia inhibition is likely to occur at low C/N ratios, which can result in pH values exceeding 8.5 causing inhibitory effects on methanogens (Matheri et al., 2017). Conversely, higher C/N can cause a deficit of N which results in the lowering of the pH of the bioreactor resulting in inhibitory effects on methanogens (Omondi et al., 2019). An optimal C/N ratio is required for effective and efficient operationalization of the AD processes (Kothari et al., 2014). Co-digestion of FW with WH in the AD system has the potential of balancing the ammonia nitrogen, C/N ratio, and alkalinity to the optimal range necessary to enhance the biomethane yield.

3.3. Biochemical characteristics

The total solids (TS) for FW and WH were 60.78 and 62.20 g/L of samples. The Volatile Solids (VS) in FW and WH were 54.12 g/l and 44.68 g/L respectively. A higher VS/TS ratio implies a higher organic content which is desirable for biogas and methane production (Li et al., 2013). Food waste contained higher VS compared to WH an indication that the FW was rich in organic matter content that could easily be converted to biogas as reported in previous studies (Zhang et al., 2013). The high VS/TS ratio is an indication of rich organic content hence FW can easily and rapidly hydrolyze. The findings of the current study were that FW had VS/TS of 89.05% which compares with the values that were obtained by Pagliacci et al., 2016 and Kawai et al., 2014 who had values of 95% & 93% respectively. However, the VS/TS for WH was 71.83% which compares with the values that were obtained by Hernandez-Shek et al., 2016, Gupta et al., 2016, and Brown et al., 2020 which were 76.54, 73.08, and 70.6 respectively. Total Chemical Oxygen Demand (TCOD) concentrations in FW and WH were 170 g/L and 140 g/L, respectively, indicating high and low content of organic matter in these substrates. The SCOD for FW and WH were 85 and 60 g/l respectively. The higher SCOD in the substrates indicates a higher amount of organic matter which is readily biodegradable and may lead to an increase in biogas production (Hallaji et al., 2019).

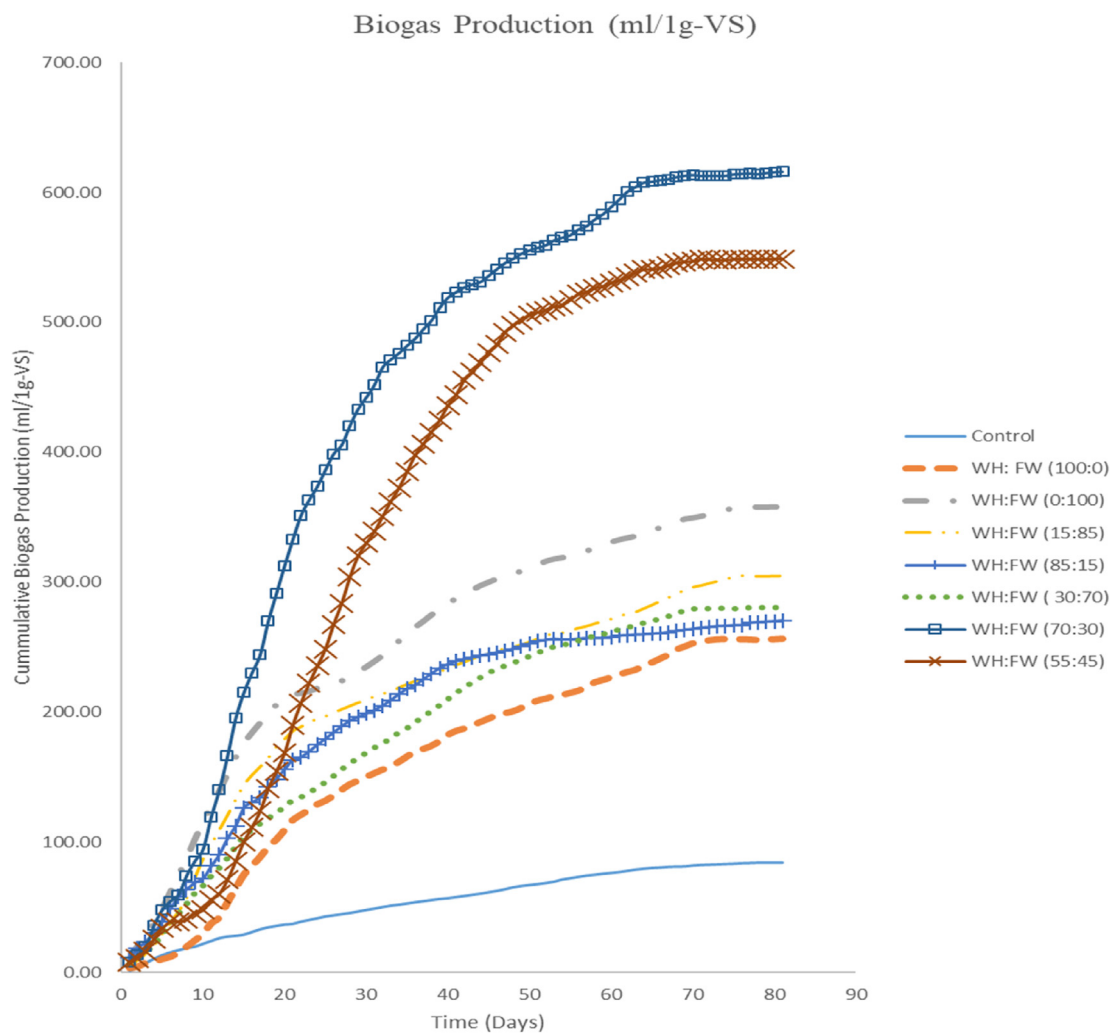


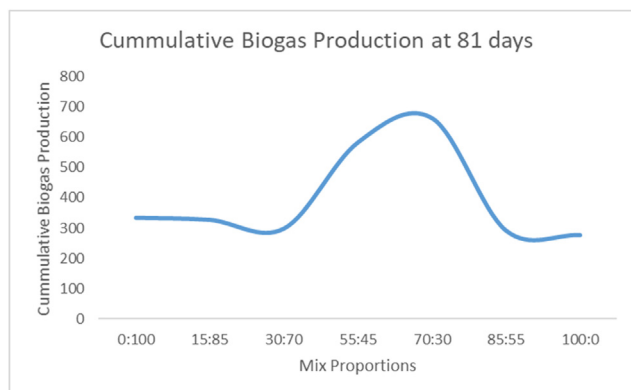
Figure 1. Cumulative biogas production.

Table 3. Cumulative biogas production.

Mix proportion (%WH: %FW)	Biogas yield (ml/1gVS)
0:100	357.85 ± 24
15:85	305.01 ± 22
30:70	280.27 ± 18
55:45	548.91 ± 35
70:30	616.01 ± 48
85:15	270.27 ± 21
100:0	256.27 ± 20

3.4. Biogas generation potential of the substrates

Figure 1 and Table 3 show cumulative biogas production for WH and FW when digested separately and when they were co-digested at different mix proportions. The cumulative biogas production for the various mix proportions at 81 days is shown in Figure 2. The experiment was conducted at Mesophilic Temperature (37 °C) for 81 days. When WH was mono-digested, it showed a slower rate of biogas production for up to 13 days. Biogas production for mono-digested WH exhibited a longer initial phase lasting up to approximately 13 days which may be attributed to the lignocellulose nature of the WH which results in reduced accessibility of the hydrolytic bacteria to the substrate during the hydrolysis stage (Cucina et al., 2021). Subsequently, WH exhibited a steady biogas production up to 81 days which gave a cumulative biogas production of 256.27 ml/1g-VS which is 30% less than the value produced by mono-digestion of FW. Co-digestion of WH with FW improved the biogas production by 5%, 9%, 15%, 53%, and 58% for mix proportions of 85:15, 30:70, 15:85, 55:45, and 70:30 (WH: FW) respectively. The statistical analytical results showed that when WH was increased from 0% to 15% in the mix ratio, the biogas production increased marginally ($p = 0.331$) showing that there was no significant difference in biogas yield ($p > 0.05$). This suggests that mixing two or more substrates in co-digestion is not a guarantee to achieve higher biogas yields than the original/parent materials in the mix. There has to be an optimal ratio. However, when the proportion of WH was increased to 30% there was a significant improvement in the biogas yield ($p = 0.016$). The results showed that the optimal ratio for the improvement of biogas yield was when WH proportions increased to 55% and 70% ($p = 0.00$). WH is lignocellulose biomass and contains lignin and cellulose which required a longer time to break down to carbon for the methanogenic bacteria to produce methane (Yadav et al., 2016). The findings are consistent with the results of similar lignocellulose-containing substrates reported by Garcia et al., 2019. Similar lignocellulose materials previously studied were sunflower husks and giant cane straws (Garcia et al., 2019). This is an indication that hydrolytic enzymes had limited access to the carbohydrates because of lignin which is strictly linked to cellulose and Hemicellulose

**Figure 2.** Cumulative biogas production at 81 days for various mix proportions.**Table 4.** Theoretical methane potential reported by other authors.

Author	Theoretical Methane Potential (TMP) (ml/g-VS)		
	FW	WH	Author
This study	766.70	412	This study
Li et al., 2013	725	349	Brown et al., 2020
Wang et al., 2017	660.5	429.9	Ledur Kist et al., 2018
Ding et al., 2021	494		

WH-Water Hyacinth, FW-Food Waste.

(Fernández-Cegrí et al., 2012). WH, a lignocellulosic material consists basically of cellulose, hemicellulose, and lignin which results in the creation of a very resistant and recalcitrant biomass structure that makes hydrolysis rate-limiting in the AD process (Sawatdeenarunat et al., 2015). Elemental analysis of the substrate shows the value of the C/N ratio of WH at 10.53, which could be a contributory factor to the ammonia inhibition hence low biogas production in mono-digestion of WH. This observation is consistent with the observation made by Wang et al., 2012 when he carried out anaerobic digestion of wheat straw which is lignocellulosic biomass that produced less inhibition at a higher C/N ratio of 25, 30, and 35 as compared to a lower C/N ratio of 15. The optimization of C/N was obtained when the WH was co-digested with the FW.

On the other hand, FW exhibited rapid biogas production in the initial stage of the experiment in the initial 20 days which could be linked to the high Volatile Solids (VS) content. Thereafter there was a lag in biogas production which could be attributed to the production of volatile fatty acids during hydrolysis and acidogenesis that reduced the pH and inhibited the activities of the methanogens (Algapani et al., 2016). Food waste had a high VS/TS ratio (organic matter content) at 89%, and a great amount of volatile acids is produced. The volatile fatty acids accumulate inside the reactor, adversely impacting the buffer's capacity and decreasing the pH (Yadav et al., 2016). The initial period lasted up to 32 days after which gas production resumed. This might be due to the use of volatile acids and the reformation of the bicarbonate buffer during the methane production process (Omondi et al., 2019). After 81 days, the cumulative gas production from FW was 357.69 ml/1g-VS. The co-digestion of FW with 70% and 55% of WH improved the biogas production by 58% and 53% respectively.

The mixture of WH: FW (70:30 and 55:45) exhibited the most stable biogas production throughout the period this could be attributed to the complementing effect of food waste and water hyacinth on the process performance parameters. The co-digestion of the substrates could have improved the process parameters by reducing acidity caused by the high C/N ratio, reducing the inhibitory range, and increasing the buffer capacity (Fernández-Cegrí et al., 2012). Co-digestion of the lignocellulose material with highly biodegradable organic matter improves the bio-methane potential and microbial activity (Cucina et al., 2021). The mix ratio of 70:30 (WH: FW) was found to be the optimum since it gave the highest biogas production of 616.01 ml/1gVS.

3.5. Biogas quantification and kinetic modeling

3.5.1. Theoretical bio-methane potential (TBMP)

The theoretical bio-methane potential (TBMP) of the WH sample calculated from the elemental composition using the Boyles equation (Eq. 3) was 412.28 mL CH₄/g VS; this showed that the biodegradability index of the WH was 53.13% which highlights the low biodegradability of WH due to its lignocellulosic nature (Brown et al., 2020). In their studies determined the biodegradability index of the WH be 37% and 30% respectively. The difference can be attributed to the differences in the composition of WH samples from the different locations and ages of the WH used in the experiment. It is also worth noting that the methane potential of lignocellulose mass can vary depending on particle size and pre-treatment (Wandera et al., 2018).

Table 5. Gompertz model parameters for WH: FW ratios.

Reactor	WH: FW	G ₀ (mL/gVS)	R _{max} (mL/gVS)	λ(days)	R ²
D1	100:0	257.9	5.409	2.599	0.9867
D2	0:100	356.6	10.01	1.052	0.9953
D3	15:85	290.4	8.329	1.75	0.996
D4	85:15	266.5	6.199	2.456	0.9963
D5	30:70	292.7	7.035	1.956	0.9952
D6	70:30	607.7	18.19	3.777	0.996
D7	55:45	560.8	15.77	2.074	0.992

The estimated theoretical bio-methane potential (TBMP) from food waste was found to be 666.70 mL CH₄/g VS this gave a biodegradability index of 46.65% which is an indication of inhibition effect during the methanogenesis stage due to the formation of volatile fatty acids likely to lower the pH of the bioreactor (Ayobami et al., 2021). The TBMP of FW was higher than WH which was an indication of the availability of highly biodegradable organic compounds (Li et al., 2013). The results from this study showed that the two substrates have a good capacity for biogas production. The calculated methane potential from the C/N ratio is useful in determining the best substrate for methane production. Table 4 shows the results of TMP from various types of research that have been carried out and how the findings compared to the current study. The variations in the biomethane potentials of the substrates could be attributed to several additional variables, which include; seasonal variation in the biochemical composition of the substrates and biomass maturity or age of water

hyacinth at the time of sampling. The characteristics of FW tend to vary geographically from continent to continent and collection source (Fisgativa et al., 2016). Fisgativa also observed that the characteristics of substrates are also affected by season variability. Tovar-Jiménez et al., (2019) also pointed out that the characteristics of WH varied significantly depending on the geographical location. This creates difficulties in comparing biomethane yields across the literature (Brown et al., 2020). However, the current study findings are comparable to existing literature.

3.5.2. Modified Gompertz kinetic model

In Table 5, we can see the model's kinetic parameters R_{max}, G₀, and Coefficient of determination R² values ranged from 0.992% to 0.996% for different FW: WH ratios, while they were 0.995% and 0.987% for FW and WH alone, respectively. This demonstrated that the model accurately reproduced experimental data, as the predicted values were in good agreement with the observed data. The RSME was between 2.663 to 9.359 which compares to the values that were reported by Paritosh et al., 2017. The model's good fit to the data was indicated by the low RSME values. From this study, the R_{max} values varied from 5.409 to 18.19 mL/g VS. The highest rate of bio-methane output was observed for the 70:30 mixtures. The model's projected values for varying FW/WH ratios are presented in Figure 3. The model was simulated to show how the experimental data fitted with the predicted values. Figure 4 shows the model simulation for biogas production. According to data in Table 5, when WH levels rose, so did lag times. According to Omawah 2020, lag time is an indicator of specific methanogenic activity (SMA) of the microorganisms and the least amount of time desired for the start of successful methanogenesis in a

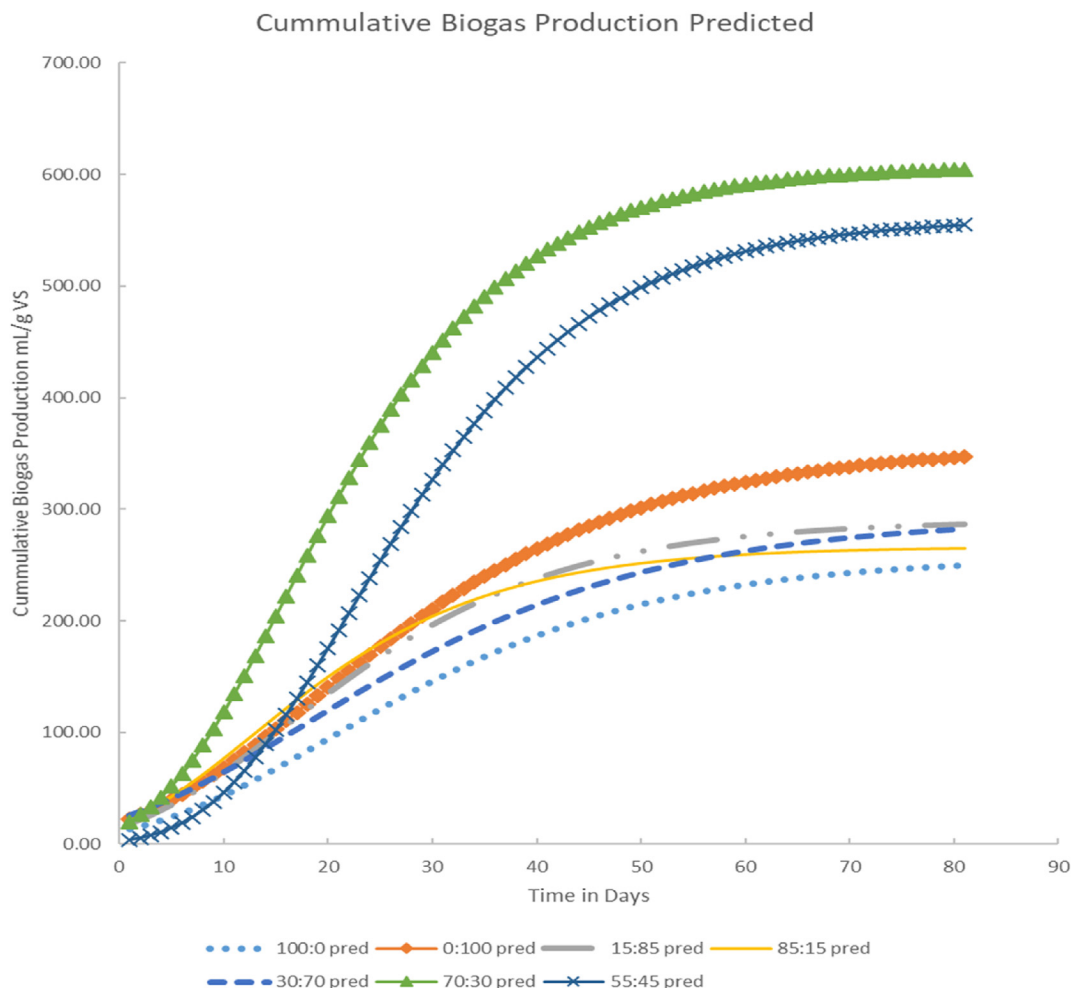


Figure 3. Cummulative biogas production simulated from Modified Gompertz model.

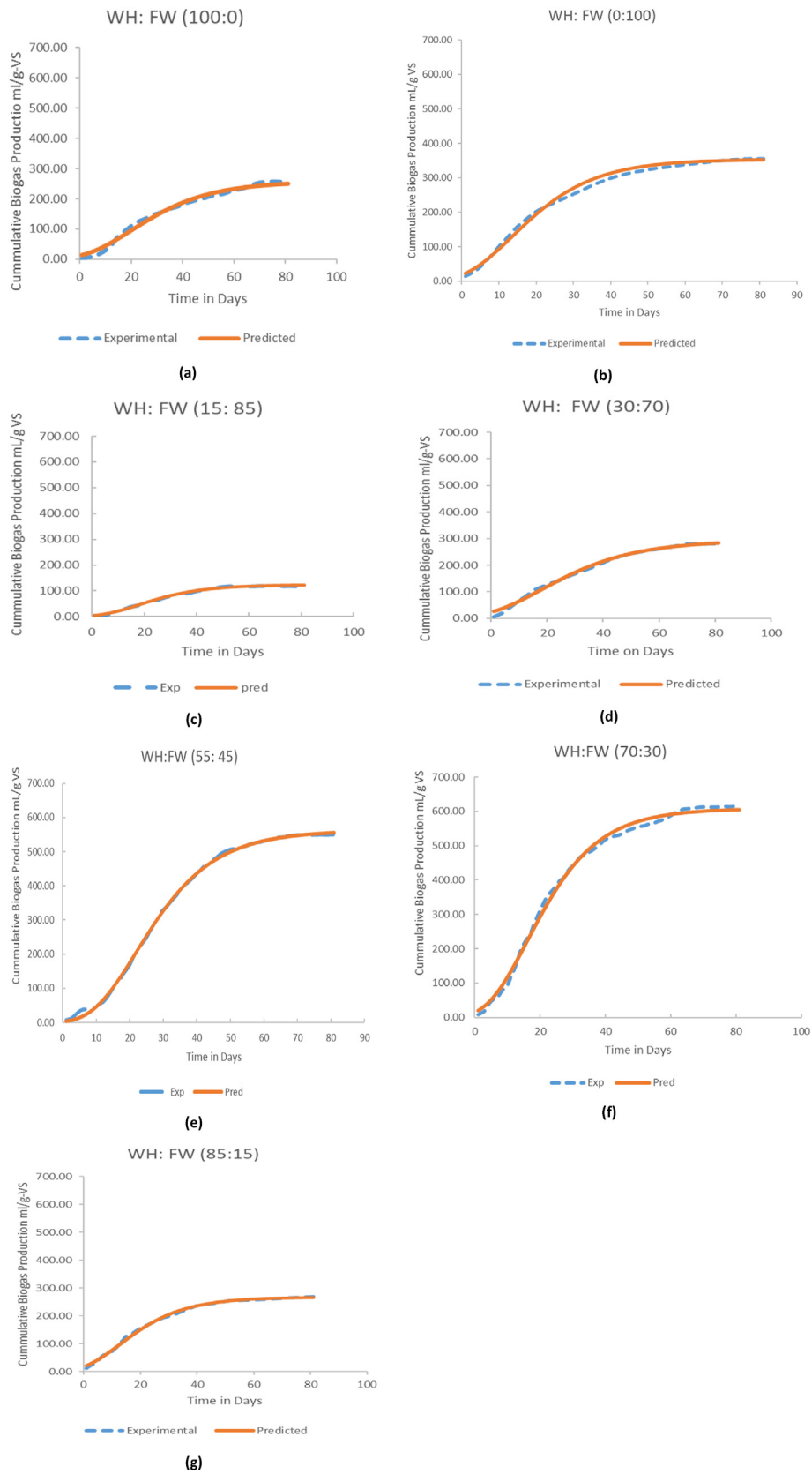


Figure 4. Plates a, b, c, d, e, f, and g showing the model simulation of biogas production for the various mix proportions of WH: FW of 100:0, 0:100, 15:85, 30:70, 55:45, 70:30, and 85:15 respectively.

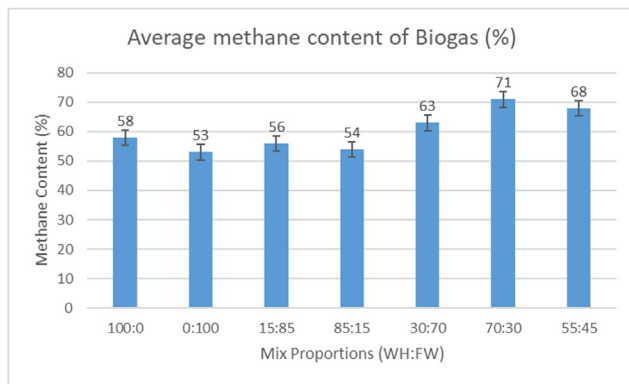


Figure 5. Methane content.

Table 6. Methane composition in biogas at different mix proportions.

Substrate	WH: FW	Average methane content of Biogas (%)
D1	100:0	58 ± 1
D2	0:100	53 ± 4.35
D3	15:85	56 ± 1
D4	85:15	54 ± 2.65
D5	30:70	63 ± 1
D6	70:30	71 ± 1.15
D7	55:45	68 ± 5.29

reactor. The enormous amounts of easily digestible organic materials and naturally occurring microorganisms in FW allow for quick biogas production once digestion has begun. However, FW typically suffers acidification because of the synthesis of excessive intermediate products like volatile fatty acids, which is caused by the high C/N ratio (Ghasimi et al., 2009). It is necessary to co-digest FW with lignocellulosic biomass like WH for correction of C/N because the volatile fatty acids impede further biogas production as anaerobic digestion advances (Pramanik et al., 2019). According to Table 5, the maximum rate of biogas production (Rmax) increased from 5.409 to 6.199 ml/g-VS when FW was increased by an additional 15%. D6 and D7 might be stated to have enhanced specific methanogenic activity and, by extension, enhanced biogas output, because Rmax is an indicator of the number of methanogens present in a reactor. The results showed that the maximum biogas yield for mono digestion of WH was 259.9 ml/g-VS, but that adding 15% FW to the WH significantly improved the results, increasing the biogas yield to 266.5 ml/g-VS. Maximum biogas production potential increased by 3%, 11%, 12%, 53%, and 57% between D1 and D4, D3, D5, D7, and D6, respectively. Because of the change in substrate characteristics caused by the co-digestion of WH and FW, the biogas output increased in D6 and D7, suggesting a novel metabolic pathway may have occurred, leading to better degradation (Yoon et al., 2017). Hydrolysis may be a rate-limiting mechanism in WH, which may explain why Rm is low in D1. WH contains a high concentration of lignin (Hernández-Shek et al., 2016). To simulate his experimental results, Panigrahi et al. (2020) used three distinct models for co-digestion of FW with processed yard waste. With the Modified Gompertz Model, he found the smallest discrepancy (3.7–15.4 percent) between predicted and experimental biogas yield. The co-digestion of food waste and wastewater sludge was simulated using the modified Gompertz model and the first-order kinetic model by Yoon et al., 2017; the former showed a higher correlation value (0.92–0.99).

3.6. Gas quality observation

The mean methane content of biogas from the various reactors is shown in Figure 5. The main source of energy in biogas is the methane

Table 7. Methane composition for WH and FW from other studies.

Substrate	Average methane content of Biogas (%)	Author
WH mono digestion	58	This study
FW Mono digestion	53	This study
Co-digestion of FW with WH	71	This study
WH (Mono digestion)	49–53	Njogu P, et al., 2015
FW (Mono digestion)	55	Qiao W, et al., 2011
FW (mono digestion)	58	Zhang C, et al., 2013
FW co-digested with Cattle manure	62.3	Zhang C, et al., 2013
WH mono digestion	57.5	Hernández-Shek M. A, et al., 2015
WH co-digested with fruit & vegetable waste	60.5	Hernández-Shek M. A, et al., 2015

WH-Water Hyacinth, FW-Food Waste.

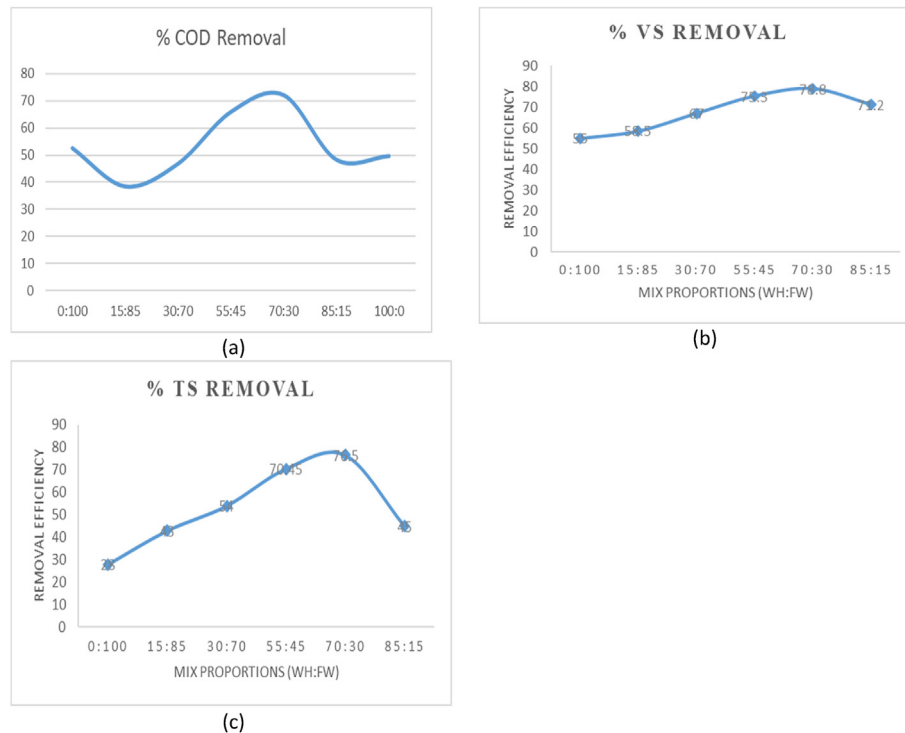
component, this shows that the more the methane content the better the gas quality is. The percentage of the methane composition is shown in Table 6. From the results, it can be observed that biogas from D2 had the least percentage of methane. This is due to the rapid hydrolysis of the readily available organics in the FW which contributes to the acidification of the digester. This contributes to lower activity and growth of the methanogens. A similar observation was reported by (Rahman et al., 2021) in his study when he mono-digested Kitchen Waste (KW) where he reported that due to higher food to microorganism ratio resulted in lowering of the methanogenic activity. However, from this study, it was noted that co-digesting FW with WH improved the percentage of methane in biogas. Undigested protein is abundant in WH and may contribute to methanogenesis (Omondi et al., 2019). The presence of protein facilitates methane production due to the synergistic impact of co-digestion which increases the buffering capacity and improved the C/N ratio (Kafle et al., 2013). From the results D1, D3, D4, and D5 had methane content of 58%, 56%, 54%, and 63% respectively. The content of methane was highest in D6 and D7 at 71% and 68% respectively which was an indication of the buffering capacity from the ammonia broken down from the protein in WH. The methane percentage from WH was 58% this could be attributed to the inhibition caused by the excess ammonia due to the low C/N ratio of the WH. High amounts of ammonia result in the inhibition of the methanogenic activity of acetotrophic methanogens which are very sensitive to ammonia hence lowering methane production (Rahman et al., 2021). These results are consistent with the observations made by (Wang et al., 2012), when he co-digested rice straw, dairy manure, and chicken manure it was observed that there was an increased methane content under mesophilic conditions. Table 7 shows the percentage methane content for the two substrates from various studies.

3.7. The removal efficiency of the organic matter

Table 8 shows the removal efficiencies of organic matter at different mix ratios of the substrates. For all the mix substrates, the initial TS was maintained at 60 g/L (6% of the DM). The rate of TS removal for mix ratios of WH: FW of 70:30 and 55: 45 was up to 76.5% and 70.45 respectively. However, it is noted that the TS removal rate is lower in the case of mono-digestion of the substrates, this could be attributed to the acidification of the reactor hence inhibiting the activities of the methanogens in the case of the FW. For the case of mono-digestion of the WH, the lower removal rate could be attributed to the lignocellulose nature of the substrate which inhibits hydrolysis. From the data, it was also seen that the rate of removal of the VS follows the same trend as that of the TS. For the mix proportions of WH: FW of 70:30 and 55: 45 the removal rate was 78.8 and 75.3% respectively. As for the COD, as the proportion of WH increases the rate of degradation of COD is increased gradually to

Table 8. Variations of TSs, VSs, and COD before and after the experiments under different mix proportions.

	WH: FW (100:0)	WH: FW (0:100)	WH: FW (15:85)	WH: FW (85:15)	WH: FW (30:70)	WH: FW (70:30)	WH: FW (55:45)
COD Initial (g/L)	60	85	65	78	74	68	72
COD Final (g/L)	30.24	40.375	40.04	40.326	39.442	18.972	24.624
COD removal (%)	49.6	52.5	38.4	48.3	46.7	72.1	65.8
TS Initial (g/L)	60	60	60	60	60	60	60
TS Final (g/L)	39	43.2	34.2	33	27.6	14.1	17.73
TS removal (%)	35	28	43	45	54	76.5	70.45
VS Initial (g/L)	34.97	54.12	48.5	46.43	49.65	50.44	50.23
VS Final g/l (g/L)	13.88	24.35	20.13	13.37	16.38	10.69	12.41
VS Removal (%)	60.3	55	58.5	71.2	67	78.8	75.3

**Figure 6.** Plates a, b, and c showing the % removal efficiency of COD, VS, and TS respectively.

72.1% and 65.8% for the mix proportions of WH: FW of 70:30 and 55: 45 respectively. The removal rate for FW only was decreased to 52.9% due to the presence of complex organic compounds such as lipids which are not easily biodegradable hence resulting in a low rate of COD removal. When only WH was used, the rate of removal of COD was also low and this could be attributed to the ammonia nitrogen inhibition. The greater removal efficiency of co-substrates during co-digestion compared to mono-digestion further supports the improvement of organic matter treatment in substrate pairs. The improvement is most likely due to the optimization of basic organic nutrient compositions, such as the C/N ratio, in WH, which is lignocellulosic biomass (Xie et al., 2017). Because lignin is tightly coupled to cellulose and Hemicellulose, hydrolytic enzymes had limited access to the organic part of the biomass in WH during mono-digestion (Fernández-Cegr et al., 2012). WH, a lignocellulosic substance, is mostly composed of cellulose, hemicellulose, and lignin, resulting in a very resistant and recalcitrant biomass structure that makes hydrolysis rate-limiting in the AD process (Sawatdeenarunat et al., 2015). Food waste has a high VS/TS ratio (organic matter content) of 89 percent, which means it produces a lot of volatile acids. Inside the reactor, the volatile fatty acids build up, reducing buffer capacity and lowering pH (Yadav et al., 2016). Co-digestion of the substrates may have enhanced process parameters by reducing acidity produced by a high

C/N ratio, decreasing inhibitory range, and improving buffer capacity (Fernández-Cegr et al., 2012). The rate of organics removal and microbial activity are improved when lignocellulosic material like WH is co-digested with highly biodegradable organic materials like FW (Cucina M, et al., 2021). From the removal rates of the COD, TS, and VS it can be concluded that anaerobic co-digestion of the FW with WH at a mix proportion of WH: FW of 70:30 gave the best improvement in treating the two organic wastes. Figure 6 shows the organic removal efficiency plots.

4. Conclusion

In this study, the impact of co-digestion of FW and WH at different proportions on the performance of AD on treatment efficiency of organic matter and biogas production was evaluated through laboratory tests.

- The experimental findings prove that the anaerobic co-digestion of WH with FW enhances biogas production compared to mono-digestion. The co-digestion of the WH with FW improved the biogas production by 5%, 9%, 15%, 53%, and 58% for mix proportions of 85:15, 30:70, 15:85, 55:45, and 70:30 (WH: FW) respectively.
- The maximum Biomethane Production Potential (BPP) increased for the substrates upon co-digestion of WH and FW. The BPP increased by

3%, 11%, 12% 53%, and 57% respectively for mix proportions of 85:15, 15:85, 30:70, 55:45, and 70:30 (WH: FW).

- The R_2 values for the modified Gompertz model were higher (0.992–0.9963) which was an indication that the model fitted well with the experimental and predicted data.
- The organic matter removal efficiency of the co-digested WH: FW improved with the percentage removal of VS solids being 55%, 58.5%, 60.3%, 67%, 71.2%, 75.3%, and 78.8% respectively for mix proportions of 0:100, 15:85, 100:0, 30:70, 85:15, 55:45, and 70:30
- The methane content improved from the co-digestion of the substrates from the results mix proportions of 100:0, 15:85, 85:15, and 30:70, giving methane content of 58%, 56%, 54%, and 63% respectively. The content of methane was highest in mix proportions of 70:30 and 55:45 at 71% and 68% respectively.

Declarations

Author contribution statement

William W. Oduor, Simon M. Wandera, Sylvia I. Murunga & James M. Raude: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

Data will be made available on request.

Declaration of interest's statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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