

Co-digestion of wastewater treatment sewage sludge with various biowastes: A comparative study for the enhancement of biogas production

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Abstract

The objective of the present study is to improve the anaerobic digestion of sewage sludge via co-digestion with biowastes. In the study, anaerobic co-digestion of sewage sludge and six organic waste materials as possible co-substrates for enhancement of bio-methane potential were carried out. Furthermore, the possible impact of the co-digestion on digested sludge content in terms of volatile solids (VS), Chemical oxygen demand (COD), and total solids (TS) were examined. Firstly, physicochemical characterization of sewage sludge as the anaerobic digestion feedstock was carried out before anaerobic digestion to obtain their composition. Characterizations such as proximate and ultimate analysis were carried out using a Hach spectrophotometer, a laboratory oven, and a furnace. The characterization feedstock results showed that the sludge contains a reasonable amount of COD, organic matter, TS, and VS along with the inhibitory compounds which shows potential for a high yield of methane in biogas production, as long as the stability of anaerobic digestion is maintained throughout the process. The results show that the sewage sludge has great methane potential during biogas production quantified as 28.6 g CH₄/kg feed. When compared to sewage sludge mono digestion, biomethane yield was increased by 3–6 times when thick co-substrates were used. Although high solid content co-substrates generated significantly more methane gas, they also increased the risk of organic overloading. Co-substrates such as molasses, food waste, animal manure, and fresh produce waste performed well at 25% co-digestion ratios. Furthermore, all other solid co-substrates co-digestion resulted in increased VS and COD residuals in digested sludge, with exception of manure. Most of the liquid co-substrates studied here, on the other hand, had a significant synergistic effect, enhancing the removal of TS, VS, and COD during anaerobic digestion.

Keywords: Brewery Spent Grains Biodegradation; Municipal Sewage Sludge; Methanogenesis; Anaerobic Co-Digestion

1. Introduction

The Republic of South Africa intends to strengthen its renewable energy sources to meet high energy demands while reducing its carbon footprint [1]. The development of alternative

energy resources will assist the country in lowering the high costs of imported petroleum oil. As a result, bioenergy generation technology has the potential to expand and diversify South Africa's energy supply, reducing the country's reliance on dwindling coal reserves and increasing its energy supply. Self-sufficient energy WWTWs have been investigated for their potential to reduce operating expenses, energy consumption, and carbon emissions [2], [3]. It has been discovered that in waste beneficiation, the process further renders WWTWs self-sustaining and energy sufficient. South Africa has a high degree of urbanization and industrialization that highlights major cities are witnessing increased population growth. In these cities, there is a rapid growth of infrastructure to meet the needs of its citizens. As a consequence, the production of biowaste materials has sporadically increased. Agricultural, urban, and industrial effluent biowaste products pose significant risks to human health. The Ministry of Health, South Africa has also expressed concerns about their disposal because they attract disease vectors such as mosquitoes, flies, and rats to sites in which sites are located [4].

Since biowastes contain organic matter that can strategically be converted to energy, literature has reported numerous studies on the beneficiation of biowastes and ways to optimize the process. Morale-Polo et al. have successfully recovered energy from fresh produce wastes, Charis et al [5] and Ferrase et al., have demonstrated that biomass can be converted into biochar which can be used as a precursor of bioenergy production. Similar studies have been reported by Ragauskas et al., [6], De Mes et al., [7], and Jackowski et al., [8]. Amongst other techniques reported, anaerobic digestion (AD) has received much interest because it is both a waste treatment technology, which enhances environmental quality, and a sustainable energy-producing technology unlike the energy-intensive process such as thermochemical conversion [9], hydrothermal gasification [10] & catalytic pyrolysis [11]. Anaerobic digestion is a methanogenic degradation of organic matter under oxygen-free conditions that involves a diverse consortium of anaerobic microbes that convert organic matter into biogas [12]. The AD process has gained interest because it has successfully employed in a wide range of biowaste effluents such as municipal sludge, animal manure, industrial sludge, and agricultural amongst others [12]. This approach significantly solves the issues associated with the disposal of waste and it is more environmentally friendly than landfills, incineration or discharge [13].

The anaerobic digestion mechanism entails the sequential decomposition of organic substances by mutually reliant anaerobic microorganisms. This metabolic route is comprised of four basic mechanisms: disintegration & hydrolysis, acidogenesis, acetogenesis, and methanogenesis. The initial phase is hydrolysis where fermentative bacteria break down the complex organic polymer i.e. lipids, protein, and carbohydrates, into simple dissolved compounds; amino acids, sugar monomers, and long-chain fatty acids respectively [14]. The acidogenic bacteria thus ferment hydrolyzed soluble substrates into VFAs, producing a moderate amount of CO_2 , H_2 and acetic acid [15]. The acetogenesis stage oxidizes the intermediate metabolites to acetate and H_2 by homoacetogenic and the H_2 producing bacteria, respectively [16]. In methanogenesis, acetolactic & hydrogenotrophic methanogens convert acetate and H_2 into CH_4 . A balanced microbial population is required for the optimal operation of an AD process. For an AD process to be stable, substrate conversion and integration must be effectively carried out during each biological phase of the process [17].

Anaerobic digestion waste treatment applications have considerable benefits that go beyond simple waste management. Bachman et al., [18] reported that the advantages of AD include the introduction of alternative energy source, as well as environmental sustainability by conservation of resources i.e. fossil fuels, as a substitute for the generation of energy [19]. Other environmental benefits of anaerobic digestion include improved disease control, air quality, odour reduction, limiting sludge production and a reduction in greenhouse gas emissions [20]. The major challenge with anaerobic digestion to date is insufficient biogas produced [21], foaming with the digester [22], too low/high organic loading rates [23], stability of the digestate and the lack of knowledge in AD reaction mechanisms [24]. These challenges are often associated with insufficient process control. Foaming arises from an imbalance of the reaction rates for those microbial steps promoted by acidic conditions, causing operational issues including; reduced biogas production, damaging the process equipment by obstruction of valves and rupture of digester tanks. Moeller et al., [25] discovered that an ammonium-nitrate-urea fertilizer solution is a viable reagent for foam reduction for the sugar beet co-digestion process. However, this inhibits the production of biogas because of the presence of ammonia.

Khainthola et al., [26] conducted a review on the enhancement of biogas and it was highlighted that co-digestion increases the quantity and the quality of biogas production. According to Rabii et al., [27] co-digestion involves simultaneous digestion of two or more substrates with the main goal of biogas enhancement by creating a more controlled environment to allow optimization of parameters that favors the AD process. Co-digestion with a suitable co-substrate has the potential to address insufficient biogas production, stability of digestate, foaming and over-acidification [28]. Few studies have explored anaerobic co-digestion of specific organic wastes as co-substrates to optimize biogas production, however none have conducted a comparative study of various biowastes. The purpose of this research is to determine the effect of anaerobic co-digestion on methane production from a sewage sludge and various biowastes combination. If the augmentation is successful, this study will quantify the effect and evaluate its mechanism. This research starts by evaluating the physicochemical analysis as a guideline, particularly with respect to the physicochemical parameters that boost or hinder biogas production. The influence of using multi-feedstock in various co-digestion systems for the enhancement of biomethane is also examined.

2. Materials and method

2.1. Sample, study area, and sampling method

Base Substrate and Sampling: The sewage sludge was collected randomly, within 6 months from two municipal WWTWs in Gauteng Province, South Africa, Johannesburg & Pretoria area as a case study. The sample points selected were primary sludge sampling point. This was selected because these points are representative of the cross-section of the entire flow thus obtaining a well-mixed sample. The standard sampling method used was a grab sampling technique from anaerobic digestion influent streams. The collected samples were stored in a cold room laboratory fridge set at 4 °C before analysis. Samples were set aside for physicochemical tests & to conduct a metagenomics analysis on the AD feedstock.

“Chemicals: The chemicals used for ADs tests were hydrochloric acid (HCl, 99%), sodium hydroxide (NaOH, solid pellets) and sodium bicarbonate (NaHCO₃, 99%), Zinc Chloride (ZnCl₂, solid) and Potassium Hydroxide (KOH, solid pellets). All chemicals were of analytical grade and purchased from Merck, Qatar.”.

Organic co-substrates Sources: The co-substrates were collected randomly from the following sources;

- Dairy Thick (DT) waste, sourced from Clover
- Brewery-spent grains (BSG) were provided by SAB Miller (InBev) Rosslyn Pretoria.
- The sugar cane molasses (M) was collected from Northern works provided by Durban Sugar Mill.
- Animal manure (AM) mixture was collected from Cavalier Abattoir, Pretoria
- Fresh Produce (FP) waste was provided by JHB Fresh Produce market, City Deep Johannesburg.
- Food waste (FW) was collected from the CSIR cafeteria, Knowledge Commons, and CSIR Pretoria.

Since there was no prior knowledge of each form of co-substrates produced on a daily basis, the random sampling method was used. It was also difficult to collect all of the different types of co-substrates generated at the source. During the tests, however, attempts were made to capture the percentage of waste produced at the market using weekly sales data from the company's website. While this method did not account for the specific form of waste produced, it did provide a fair foundation for a portion of waste in the substrate mix. Before being transported to the laboratory, the collected waste was placed in a plastic bin.

2.2. Preparation of substrates

The wastes were cut into small pieces in the laboratory (when deemed necessary), macerated and homogenized with an industrial food grinder to produce small pieces (1 mm in size), as shown in Fig. 1a. The maceration was carried out without the use of water. Before the samples were packed into labeled plastic containers and kept at -18 °C when not immediately used, a homogenized sample was taken for characterization. The frozen substrates were thawed to room temperature before use and stored at 1 °C in a cold room. To minimize the seasonal effect on substrate content, AD tests were performed within one month of substrate preparation [29]. Sludge & co-substrate characterization was carried out before the BMP test to understand the composition of the feedstock to assist in designing the experiment. These physical and chemical compositions include volatile solids (VS), and total solids (TS) according to the standard methods for the examination of wastewater as outlined in Eaton et al. [30] and ASTM E872 [30].

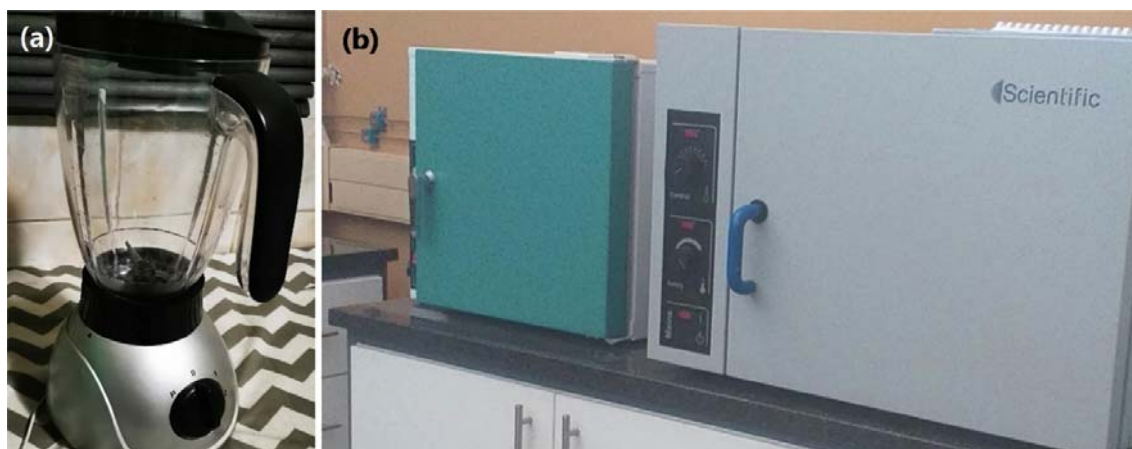


Fig. 1. (a) Small industrial food grinder to homogenize & (b) the Thermo Scientific Oven.

2.3. Proximate and ultimate analysis of organic waste

These proximate and ultimate properties are critical when selecting a feedstock for energy recovery systems. The proximate analysis includes determining the moisture content (MC), volatile matter, ash content, and fixed carbon in biowaste substrates. The aim is to determine the percentage of fixed carbon by subtracting the total of moisture, volatile matter, and ash percentage from 100. The operations were repeated three times, and the average values were provided as % TS, MC, VS, and ash content of the substrate in the composite Table 1, Table 2, Table 3. The silica crucibles were weighed before (M_1) and after (M_2) it was filled with a known mass of biowastes using a spatula or by pouring for digestate to determine MC, TS and VS characteristics. For moisture content, the crucible with the substrate was placed in a preheated Thermo Scientific Flash 6000 Oven (Fig. 1b). The substrate is dried in the oven until dry for a maximum of 24 h at 105 °C. The crucible is removed and weighed after cooling in a desiccator. The heating, chilling, and weighing processes are repeated until a stable weight of the biowaste sample is produced (M_3). Equations (1), (2) were used to calculate the % TS and moisture content, respectively.

Table 1. Sample identification and description.

Co-substrates Description								
Sludge ID	Mono Digestion	% Co-substrate	Brewer Spent Grain	Food Waste	Fresh Produce	Animal Manure	Molasses	Dairy Thick
			BSG	FW	FP	AM	M	DT
CoT	CoT-Mono	5%	BSG-CoT-5%	FW-CoT-5%	FP-CoT-5%	AM-CoT-5%	M-CoT-5%	DT-CoT-5%
		25%	BSG-CoT-25%	FW-CoT-25%	FP-CoT-25%	AM-CoT-25%	M-CoT-25%	DT-CoT-25%
NW	NW-Mono	5%	BSG-NW-5%	FW-NW-5%	FP-NW-5%	AM-NW-5%	M-NW-5%	DT-NW-5%
		25%	BSG-NW-25%	FW-NW-25%	FP-NW-25%	AM-NW-25%	M-NW-25%	DT-NW-25%

Table 2. Characterization of the Inoculum Parameters.

Parameters	Inoculum
pH	7.20
Total Solids (w/w %)	1.34
Volatile Solids (w/w %)	1.58
Carbon content, %	37.14
Nitrogen content, %	3.23
C/N ratio	11.50

Table 3. Composition of raw sludge and co-substates.

Substrates	Nutrients		Organic matter	Buffering Capacity	C/N Ratio	Physical	
	COD (mg O ₂ /L)	VS (%)	Organics (%)	TA (mg/kg)	C/N ratio	TS (%)	pH
Sugar Cane Molasses	362 562	59	25	31,634	16.4	42	5
Fresh Produce Waste	56 020	89	9.57	672	46.3	11	5.9
Brewery Spent grains	23 997	91	20.2	8690	25	22	5.12
Animal Manure	89 420	80	23	584	19	23	6.3
Fresh produce Waste	55 400	87	14	467	17	10	7.4
FOG (lipids)	715	68	3.3	787	10.6	4.8	7.1
Ice cream Waste	6 819	62	4	89	18.1	4	7.5
Food Waste	45 900	87	14	467	17	10	7.4
Soft drink waste	42 800	98	21	2.8	14	2.3	4.2
Dairy fermentation residues	34 600	83	17	308	9.6	4.8	4.8
Dairy thick waste	43 300	73	27	1998	23	29	6.8
Slaughter waste (Fats)	4 670	81	12	787	12	4.8	6.3
Slaughter waste (Blood, Content)	6 570	89	6	697	18	11	7.8

To determine the volatile matter in biowastes, the dried sample was further heated in a muffle furnace set to 550 °C and heated for three hours according to the ASTM E872 [31]. The crucible is taken out, cooled in a desiccator and weighed until a constant mass of M₄ is obtained. The volatile matter was calculated using Equations (3).

$$\%TS = \frac{M_3 - M_1}{M_2 - M_1} \times 100 \quad (1)$$

$$\%MC = 100 - \%TS \quad (2)$$

$$\%VS = \frac{M_3 - M_4}{M_3 - M_1} \times 100 \quad (3)$$

$$\%Ash = \frac{M_5 - M_2}{M_2 - M_1} \times 100 \quad (4)$$

Where M_1 is the weight of the empty crucible (g); M_2 is the weight of the crucible with wet substrate or digestate; M_3 is the weight of the crucible after drying at 105 °C, and M_4 is the weight of the crucible after furnace at 550 °C. To conduct an ash content analysis, the biowaste sample was measured as previously stated in the crucible. After weighing, it was burned in a muffle furnace in the presence of air with the open crucible at 750 °C until the consistent weight was maintained. M_5 was assigned to the leftover ash after it was measured. The percentage of ash content in the biowastes' combustible components was calculated using Equation (4). The CHNS elemental compositions of nitrogen (N), total carbon (C), Sulfur (S), and hydrogen (H) were determined using the Organic Element Analyzer. The quantity of oxygen in the waste sample was calculated analytically by subtracting the sum of the fractions of total C, N, S, and H, as shown in Equation (5). The weight percentage of O was calculated by subtracting the elements from the inorganic ash.

$$\%Oxygen = 100 - \%(C + N + S + H) \quad (5)$$

2.4. Inoculum

“The efficiency of the continuous AD process can be diminished due to operational challenges and instability issues linked with inadequate start-up and a lack of suitable inoculum. As a result, the seeding process must be carried out with biologically active sludge, known as Inoculum. Inoculum is one of the most critical components in the anaerobic digestion process since it has the potential to influence methane production results. In this order, the usual process for preparing the inoculum was followed, followed by a quality check to see whether the operational parameters of the digester are of excellent quality. The most popular approach is to pre-incubated the inoculum at 35 °C for 1 to 5 days to degas and limit the influence of methane production. IBERT supplied the inoculum for both the batch and semi-continuous AD processes, sourced from an abattoir waste treatment plant.”.

IBERT Pty supplied the inoculum that was used in both the batch AD processes. IBERT Pty is a Johannesburg-based biogas manufacturing, procurement, design, and management firm. The inoculum came from a facility that processed abattoir waste. The plant runs at 37 °C, and the inoculum had a pH of 7.75. Since sludge will be digested, inoculum from AD plants that handle such waste would have been the best choice. However, the majority of biogas plants in the CoJ use non-FVWs as a substrate. As a result, only inoculum from such plants can be used.

According to Morales-Polo et al., [32] there was no substantial difference in BMP findings for the same substrate using different inoculum sources. They concluded that the degradation would continue if the inoculum had enough diverse microbial communities to cope with the substrate's disintegration. Al-Addous et al., (2018) also found that the effect of inoculum sources on the degree of biodegradation was negligible. The source of inoculum, on the other hand, may influence the kinetic rate of degradation.

2.5. Theoretical biogas potential

Based on the study conducted by Symons & Buswell, [15] the theoretical biogas potential for the substrates studied was determined using a stoichiometric equation based on the atomic makeup of the waste material. The equation assumes that all organic matter is converted into CO_2 , CH_4 , NH_3 , and H_2S . The relative predicted biomethane potential was determined for each element present by dividing the wet weight fraction by its molar mass. The qualitative BMP is reported as $\text{NL CH}_4/\text{kg VS}$.

2.6. Physicochemical characterization techniques

The characterizations of sludge reported in this study include total chemical oxygen demand (tCOD), volatile solid (VS), total solid (TS), Chemical Oxygen Demand (COD), Total Kjeldahl Nitrogen, (TKN), Total Alkalinity (TA), Total Organic Carbon (TOC), Ammonium Nitrogen ($\text{NH}_4\text{-N}$). The measurement of pH, temperature, TS & VS parameters were carried out guided by the standard examination of wastewater examination methods as indicated in Eaton et al. [30] using appropriate pH & temp meter, laboratory oven and furnace. The analysis was carried out as soon as the sludge was conveyed to the university's laboratory. The COD, TKN, TA, TOC, and $\text{NH}_4\text{-N}$ were determined spectrophotometrically by use of standard test kits (Hach-DR3900, USA), See Fig. 2. Total Organic Nitrogen (TON) is used to calculate the C/N ratio, however Hach does not have a method for directly measuring TON, one way to determine the TON of a sample is to measure the TKN and subtract the ammonium nitrogen as shown in Equation (6).

$$\text{TON} = \text{TKN} - \text{NH}_4\text{-N} \quad (6)$$



Fig. 2. Spectrophotometric analysis method using standard Hach test kits.

The COD was used to estimate potential methane instead of volatile suspended solids (VSS) as usual for organic matter reduction. The COD of methane was calculated using $1 \text{ g COD} = 0.35 \text{ L CH}_4$ as explained in Filer et al., [34].

The amount of biogas produced in a given period from any substrate through anaerobic digestion is dependent on several factors, the operating conditions includes; digester temperature, digester pH, organic loading rate (OLR), hydraulic retention time (HRT), solids retention time (SRT), digester shape and type [35]. Temperature and temperature variations are quite important in the AD process. Temperature enhances the rate of bacterial methane generation. However, because the amount of free ammonia increases with temperature, bio-digestive efficacy may be limited or even dramatically decreased [36], [37]. Thus, unheated biogas facilities perform satisfactorily quite often in areas with average temperature values of 20–35 °C and negligible variations of 2 °C/hr. The recommendation of the most appropriate retention time (HRT & SRT) is affected not only by the operating temperature but also by the type of substrate utilized [38]. Methane-producing bacteria thrive in neutral to moderately alkaline environments. If the pH falls below 6.2, the medium becomes potentially toxic to the methanogenic bacteria [39]. To grow, bacteria require an adequate supply of organic substances & nutrients as a source of carbon and energy. However, because a higher quantity of any specific item normally has an inhibitory impact, case-by-case assessments are required to establish how much of which nutrients, if any, still needs to be given [37].

Understanding the feed composition is also crucial when one is considering optimizing biogas production from any substrate. The key determinants or parameters for anaerobic digestion include; chemical oxygen demand (COD), total solids (TS), volatile solids (VS), total organic carbon (TOC), and Total Kjeldahl nitrogen (TKN). The mobility of methanogens composed in the substrate is slowly decreased as the solids content, TS, increases, and the biogas production potentially drop if the TS is too high. According to Zhao et al., [40] the optimum range of %TS in anaerobic digestion is 5–20 % depending on the feedstock. COD determines the limit of biogas that can be produced from a given substrate. TOC and TKN determine the Carbon-to-Nitrogen (C/N) ratio which is the measure of the nutritional balance of the feed. The ratio does not have any effect on the limit of the biogas that can be produced from a substrate, however, maintaining the ratio within the optimum range ensures optimum biogas production. The total alkalinity (TA), orthophosphates (PO_4^{3-}), and ammonium nitrogen ($\text{NH}_4^+ - \text{N}$) are responsible for buffering capacity of the digester, and their composition together with VFA are important in determining the pH of the digester.

2.7. Batch experimental set-up

The Eudiometer-based BMP was equipped with 500 ml & 1L Schotts bottle operating as an anaerobic digester, refer to Fig. 3. These small digesters are fed with a calculated volume of substrates & inoculum. Inoculum is mixed with the substrate to provide a consistent & controlled bacterial supply to the system. Fig. 3 indicates the anaerobic digester setup used in the system.



Fig. 3. The Digester setup in the BMP setup, & illustration of substrates & inoculum measurements.

Nitrogen gas is pumped into the system to flush the headspace of the reactor and the biogas pipelines. This is done to ensure that anaerobic condition is achieved before the experiment was started, this also helps in ensuring that the system is airtight and there are no leaks. To measure the volumetric production of biogas, Eudiometer volumetric method is used. The liquid displacement method is used in this method to calculate the amount of biogas produced. The method measures the amount of CH_4 created after eliminating CO_2 from biogas by bubbling it through a barrier solution, in this case, a NaCl 6.0 M solution. The BMP tests were performed in mesophilic temperatures, mimicking the method and environment at the WWTP. The data on biogas accumulation and composition were obtained daily utilizing the Goodspeed SA Geotech Biogas 5000 equipment. During the digestion, the biogas quality (CH_4 , CO_2 , and balance) was tested ten times. Each variety was repeated between two and four times. Biogas production from inoculum alone was also measured. Inoculum mono-digestion was used as a reference for all anaerobic digestion, thus the methane production of inoculum was subtracted from the biogas production that was measured in the digesters that contained inoculum and biowastes.

2.8. Sample ID and description

The sample was labelled with code description starting with the name of the substrates listed in Section 2.1, followed by the sewage sludge ID and then the mixing percentage of co-substrates to the base substrate (sludge). Thus the feedstock general description is defined as co-substrate-Sludge ID-mixing ratio, meaning that BSG-NW25% present Brewer spent grain-Northern Works sludge-25% co-substrate. For mono-digestion of sludge, the ID will either be CoT-Mono or NW-Mono, refer to Table 1 for more description.

3. Results and discussion

3.1. Anaerobic Co-digestion experiments

3.1.1. Physicochemical characteristic of inoculum

IBERT Pty in Johannesburg, South Africa, provided the inoculum used for the batch study. The inoculum came from a facility that processed abattoir waste. The inoculum's chemical characteristics are listed in Table 2. The plant runs at 37 °C, and the inoculum has a pH of 7.20.

Wide-ranging volatile solids (VS), C/N ratio, solids (TS), and pH values were used to describe the inoculum. Total solids were 1.34 percent and volatiles were 1.58 percent, respectively. Chen et al., (2021) conducted a study on sewage sludge, he obtained similar results using the BMP study though geographical location and the inoculum sources were different. He also reported that though nitrogen content is slightly high, the degradation would continue if the inoculum had enough diverse microbial communities to cope with the substrate's disintegration. In addition, Dioha et al., (2013) found that the effect of inoculum sources on the degree of biodegradation was negligible. The source of inoculum, on the other hand, may influence the kinetic rate of degradation.

3.1.2. Physicochemical characteristics of co-substrates

The importance of feedstock analysis in the AD phase cannot be overstated. The amount and composition of biogas produced, as well as the amount of energy in the biogas, are all calculated using general characteristics or, more specifically, the composition of the substrate (feedstock) [27]. The quality of biogas generated, particularly methane, is largely determined by the feedstock characteristics during anaerobic digestion. The primary characteristics of the individual co-substrates are collated in Table 3. In this study, the parameters of interest are TS, VS, C/N ratio, COD, TA and pH.

The difference between each co-substrate is the COD, TS and VS contents are noticed and reported in Table 3. Higher TS is noticed from the molasses, spent grain & manure commercial sample, this could be due to the nature of a substrate, manufacturing process or storage as noted. Molasses is naturally a dense brown liquid or a thick syrup made from boiling down sugar cane. The spent grains are the major by-product of the beer-brewing industry and are collected after beer extraction by the filtration process. Dry stacking manure is the most common and most practical method of manure storage for abattoir and livestock farms. The implication of the higher solids content is a greater propensity to contribute to biogas production especially in the AD processes where feed requires thickening.

To stabilize the anaerobic digestion process required C/N (carbon to nitrogen) ratio should be between 15 and 30 [43]. The C/N ratio in sewage sludge is less than ideal. Spent grain, on the other hand, is outside of the ideal range. Brewery spent grains, fresh produce waste, and food waste are lignocellulosic materials that contain a significant amount of carbon and are resistant to degradation, slowing the pace of decomposition [44]. However, when blended at 1:3 ratio, the resulting C/N has the potential to favour anaerobic digestion and thus increase methane production. It should be noted that co-substrate selection is also influenced by sourcing considerations. As mentioned before all other co-substrates are essentially waste materials. As a consequence, their properties may be subject to considerable temporal and spatial variation.

Each BMP mix was created differently depending on the inoculum and substrate concentrations to ensure that the BMP was carried out in settings that did not limit or obstruct the anaerobic digestion process. This entails changing the inoculum and test material volumes until the substrate to inoculum ratio, as well as the headspace to total solution volume, are all within the specified parameter limits. Table 4 gives the nutrient composition of the samples to assist in the design of the experiment. From the VS results, it can be predicted by

now that NW will produce the highest methane than the other two raw sewage sludge in mono-digestion experiments. In co-digestion, NW sludge combined with spent grains, fresh produce waste, or molasses is predicted to give a good enhanced methane production.

Table 4. Composition of the blends (on 1:3 ratio) sewage sludge & co-substrates for co-digestion experiments.

Raw Samples			CoT co-digestion		NW co-digestion	
Sample ID	TS (%)	VS (%)	TS (%)	VS (%)	TS (%)	VS (%)
CoT	3.1	98.51	3.1	98.51		
NW	7.36	88.64			7.36	88.64
Inoculum	0.64	5				
Spent Grains	21.71	91.51	16.91	98.51	9.04	93.58
Fresh produce	11.43	89.57	7.27	94.04	9.40	89.11
Food waste	10.24	86.59	4.67	91.76	6.80	86.83
Thick Dairy waste	29	72.81	11.75	97.25	8.7	86.62
Dairy waste 2	4.8	83.58	5.75	98.05	7.88	93.11
Manure	23.43	80.41	5.27	93.61	7.40	88.68
Molasses	41.85	58.71	6.10	95.58	8.23	90.64
Soft drink drain waste	2.28	98.45	2.70	98.48	4.83	93.55

3.1.3. Batch studies, sludge and Co-substrates

The assessments for biogas accumulation profiles along with their corresponding profiles for the blank and control assays are presented in Fig. 4, Fig. 5, Fig. 6. The units are normal litres of biogas in a mass of volatile solids content in the substrate per retention time (number of days). In this project, the methane gas is considered normalized to STP (0 °C and 101325 N/m²) and it is assumed to be a dry gas. From the triplicates, the mean specific biogas (NL biogas/kg VS) was calculated and then plotted. The initial pH of the feedstock was low during the preparation of the feed and thus buffer agent solutions were used to maintain the pH at 6.5–7.5. In this run, the digestion experiment was completed after 31 days.

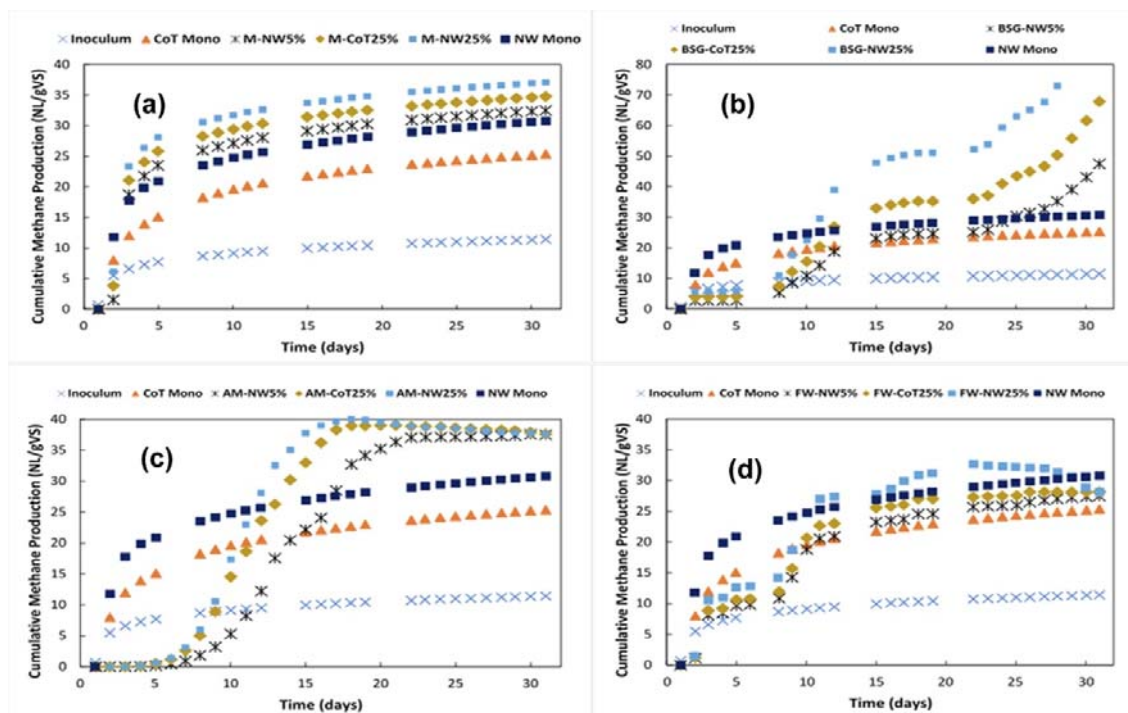


Fig. 4. “Cumulative methane production plotted against time for co-digestion of sewage sludge and biowaste as co-substrates”.

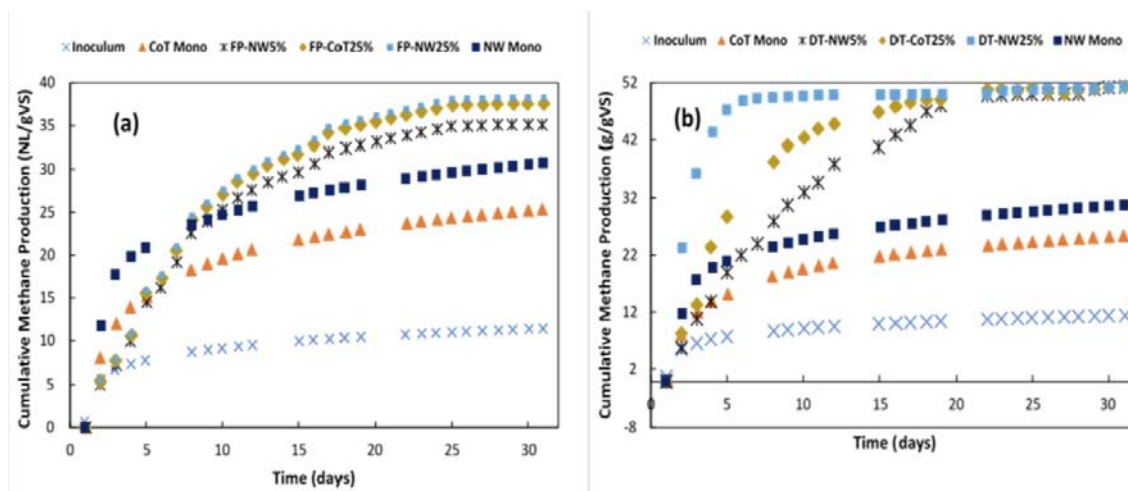


Fig. 5. Methane cumulative results for co-digestion of sludge with fresh produce and dairy thick waste.

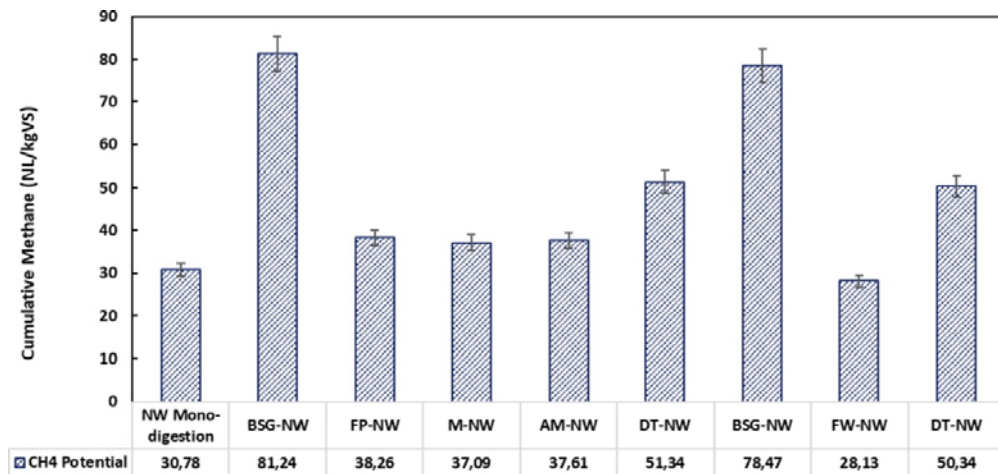


Fig. 6. Methane production of NW sludge and the co-substrates.

3.2. Co-digestion with high solid content wastes

Fig. 4a shows that as the molasses fraction increased, accumulated methane output increased for both CoT and NW co-digestion. The addition of a co-substrate from 5% to 25% (M–NW) above the optimum point increased the overall methane production. Fig. 4a, which shows an increase in production and M–NW25% molasses concentration, demonstrates the pattern. Organic loading rate, TS & COD for the anaerobic system assisted this enhancement. This is supported by the study done by Doloman et al., (2017) and Kalemba & Barbusiński, (2017). However, this was not the case for CoT sludge, the same molasses concentration (M–NW25%), achieved lower methane enhancement. This might be due to excessive development and accumulation of volatile fatty acids which resulted from a high carbon/nitrogen stoichiometric ratio. Fatty acid accumulation causes a drop in pH, which inhibits microbiological activity [47], [48]. “Fig. 5 shows the findings of cumulative biogas production profiles for mono-digestion of sewage sludge and spent grain, as well as co-digestion at a 1:3 ratio. As shown in Table 3, the C/N ratio and TS of both feedstocks were beyond the optimal range for AD when used separately, but they were inside the optimal range when combined, the same results have been reported in previous studies [49], [50].”.

The highest BMP was obtained with co-digestion of NW sludge and 25% BSG, producing approximately 80 NL/kg VS. This is a 3-fold increase when compared to the assay of sludge mono-digestion. BSG is a promising co-substrates to sludge digestion, is that it is a very good substrate for microbial growth, the high solid content of BSG reported associated with fermentable sugar presence makes it susceptible to microbial growth [51]. BSG has excellent adaptability of a shift in bacterial with different phases of anaerobic digestion pathway specified in Section 1, especially the archaeal community (methanogens) according to Bianco et al.,[52]. Additionally, it was reported previously that BSG fulfills the requirements demanded biotechnological exploitation because it is rich in protein and amino acids, which make it attractive to biogas production. Results in Table 2 show that BSG has high in organic matter & contains nutrients which are valuable compounds for good calculated energy value. Furthermore, in the microbiological interpretation co-digestion is the introduction of the hydrolytic-acidogenic species which increase the biodegradability of the blend [53]. During

the steady-state phase, it was also noted that Proteobacteria became the most common phylum, and hydrogenotrophic methanogens dominated over acetoclastic methanogens [54].

The addition of the co-substrate BSG fraction of 25% (BSG-NW and BSG-CoT) did not result in an excessive build-up of volatile fatty acids, and the produced acids had enough time to be digested, See Fig. 4b. For these co-substrate samples, the removals of TS and VS were found to be about 61 and 47%, respectively. These findings suggest that using BSG as a co-substrate would result in more biosolids development. The co-substrates had a methane capacity of around 39 & 78 NLCH₄/kg VS, respectively. As a result, the biogas production was increased in volume using co-digested samples as compared to sewage sludge alone. It was also noted that the methane production was stabilized from days 1–5, sharp increase for another 5 days until it stabilizes again, reaching the maximum methane at day 31 (Fig. 4b). The improvement in methane production could be attributed to the favorable balancing out of the C/N ratio, TS and the microbial [55], [56]. As a result of the following degradation of VFAs by methanogens, co-digestion enhances the organic matter accessible as volatile fatty acids (VFAs) and biogas productivity [57].

Approximately 37.49, 36.45 and 38.5 NL/kg feed of methane were produced from co-digestion of NW and CoT sewage sludge with different concentrations of animal manure & feeding wastes sourced from an abattoir (AM-NW 5, AN-NW25, and AM-CoT 25) mixed at 5 & 25% respectively. “These results are consistent with data from the literature [42], [58], [59]. It can be observed in Fig. 4c that methane production was stagnant from day 1 until 5, start to increase rapidly from day 6 until the production stops on day 16, and a slight decrease was observed after day 21. Jung et al.,[60] and Tyangi & Lo.,[61] substantiate that the “no production” for the first five days could be due to the intra and intermolecular hydrogen bonds that create crystal structures molecules. In addition, the presence of low soluble COD and lignocellulose in the grains increase biodegradability resistance and a slower rate of biodegradation [62].

The methane yield of all organic waste co-substrates was higher than that of merely wastewater sludge. However, when the co-digestion ratio was 5% and 25% (wt/wt), organic overloading was detected for food waste in both test periods (Fig. 4d). When the co-digestion ratio was set to 25%, biogas production for FW-NW was significantly lower. At a 25 percent co-digestion ratio, anaerobic digestion inhibition was also found with manure. Inhibition at high concentrations of food waste co-digestion ratios was connected to the accumulation of volatile organic acids, as evidenced by a low pH of less than 5 of the substrate at the end of all BMP bottles with limited methane production, similar to the findings published by Rea, [63].

Between the two sampling periods, there was a temporal fluctuation in the VS and COD of the food waste. The two food waste samples' VS and COD values differed by 20 and 65 percent, respectively. For both occasions, a co-digestion ratio of 5% was appropriate. With the fresh produce waste samples, there was also a temporal change in VS and COD content (10 and 90%, respectively). Nonetheless, even at a co-digestion ratio of 25%, the fresh produce waste samples showed no inhibition (see Fig. 5a). While the pre-collection dilution reduced the inhibitory potential, the maximum biogas output was lower than that of food waste at the same co-digestion ratio. Food waste, as well as fresh produce waste, are both

types of food waste. In other words, the composition of both co-substrates should be similar, resulting in similar methane generation. To validate the effectiveness of dilution of this co-substrate, a larger co-digestion ratio between co-substrate and sludge would be needed.

The results obtained from the BMP co-digestion of dairy thick waste (Fig. 5b) validate that the biogas production increases with the increased co-digestion ratio until equilibrium is established. It's conceivable that the production of volatile fatty acid has accelerated by the rate of hydrolysis of dairy thick waste. As a consequence, even with a low co-digestion ratio of dairy thick waste and sludge, there was no accumulation of volatile fatty acids in the system. It's also worth noting that the advantage of increasing the concentration beyond 5% was minimal.

3.3. COD removal efficiency

In anaerobic treatment, methanogenic bacteria convert organic compounds (COD) to biogas to CH₄, CO₂ & digested biomass material [64], [65]. The efficiency of biogas conversion can be validated by the removal of COD & other parameters from the digested material. Another aspect to consider is the stability of the digestate, and the purpose it is intended for. Based on the removal efficiency what recommendation can be concluded for further use i.e. composting, land enrichment, or other agriculture applications. Hence it is important to analyze the removal efficiencies in terms of TS, VS, and COD. Under operation temperatures of 20 °C and 25 °C, organic removals performed well if the COD removal efficiency is approximately 90% [66]. The BSG wastes, indicate a high COD removal efficiency and 65% TS removal efficiency, see Table 5. Dairy waste (DT), and molasses show a good COD, VS, and high TS removal efficiency. Based on these findings, it can be deduced that the COD in these biowastes is efficiently converted into methane. However, the low % removal of TS, in this case, may result in increased sludge formation and may have a detrimental impact on sludge stabilization goals. Average removal efficiency for TS and VS were found for FW & FP wastes with low COD removal. A reduced TS removal, as mentioned previously, suggests that the biowastes have high potential to result in more sludge formation. The low COD removal rate is likely caused by the low number of microorganisms in the AD operation at acidic pH conditions especially in the beginning of the process [67], [68]. In terms of removing both VS and COD, dairy thick waste also showed some promising outcomes.”

Table 5. Removal of COD, TS & VS in co-digestion of sewage sludge with high solid content wastes.

Parameters	Molasses	BSG	AM waste	FP waste	FW	DT waste
COD Removal (%)	78	90	55	65	57	79
VS Removal (%)	92	47	56	20	87	80
TS Removal (%)	65	61	68	62	59	68

These solid wastes, AM & FP, demonstrated a tendency for inadequate removal of these parameters, implying that these waste materials may result in extra sludge generation and

digester accumulation. This is also substantiated by earlier research conducted by studies conducted by Wickham et al., [69], Ji et al.,[66] & Kawai et al.,[67]. The results also confirm the validity of BMP as a screening tool for co-substrate evaluation.

3.4. Comparative study for NW and CoT sludge co-digestion

To compare the response of raw NW and CoT sludge mono-digestion and the corresponding co-digestion, bar graphs are presented in Fig. 6, Fig. 7. The co-substrates listed on the x-axis, and the corresponding accumulated methane is presented on the y-axis. Since it was presented in section 3.1 that 25% co-substrate concentration is recommended for sludge co-digestion, the results in Fig. 6, Fig. 7 are for 25% co-substrate-sludge blend feedstock. From both Fig. 6, Fig. 7, it can be observed that the feedstock modification by blending with co-substrates has improved the AD rate and reduced the inhibitory effect. Most of the selected co-substrate achieved an increased methane yield, however, the highest methane potential was obtained with co-digestion of molasses, spent grain, dairy waste and fresh produce. As expected, the mutual characteristic of all these co-substrates is the physicochemical properties such as high carbohydrates (glucose) or protein and the absence of inhibitory compounds such as H_2S and ammonia. These results are in agreement with numerous literature that shows that the addition of carbohydrate and/or protein to sludge enhances the production of methane [70], [71]. The addition decreases LCFA inhibition and improve the growth of methanogenic archaea to enable rapid recovery of digester performance [72].

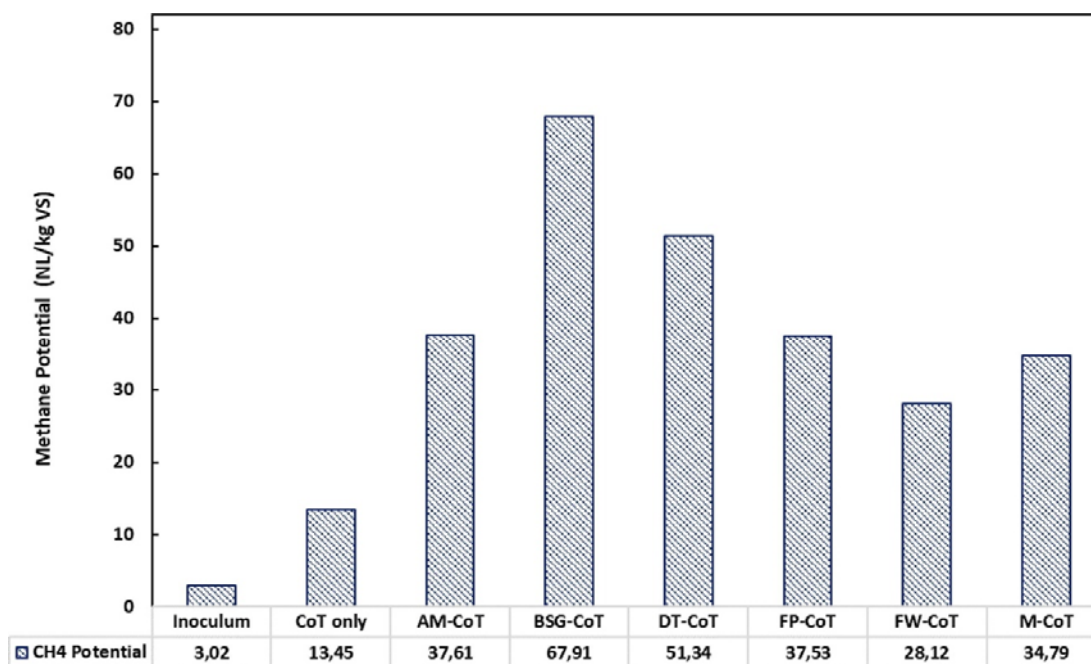


Fig. 7. Methane production of CoT sludge and the co-substrates.

Fig. 6 shows the improvement in methane production, from 30.78 NL/kg VS mono-digestion of NW sludge to almost 80 NL/kg VS for BSG co-substrates with 25% concentration. This is followed by DT co-substrate, this was however expected since the 25% blend has successfully balanced out the C/N ratio, TS, and the microbial community as mentioned previously. The co-digestion increases the organic matter available as volatile fatty acids (VFAs) and the

biogas productivity, because of the subsequent degradation of VFAs by methanogens [57]. Furthermore, in the microbiological interpretation co-digestion is the introduction of the hydrolytic-acidogenic species that increase the biodegradability of the blend [50], [53]. During the steady-state period, it was also observed that Proteobacteria became the most common phylum, and hydrogenotrophic methanogens prevailed over acetoclastic methanogens [54]. From the microbial community richness analysis done discussed previously, it was shown that NW sludge presented a higher richness of favourable methanogens. In general, higher community richness indicates more ecological stability and resistance to toxins in the AD process.

The results of cumulative methane yield of mono-digestion of CoT sludge and co-digestion of substrates are shown in Fig. 7. It can be noticed from the results that, similar to NW there was also a significant increase in methane production when adding a co-substrate to CoT sewage sludge. Different co-substrates can alter digestion circumstances, affecting microbial diversity and, as a result, changing methane generation performance. Due to the high fraction of VS on co-substrates, balanced C/N ratio, and favourable TS, temperature & pH conditions, the activity of the methanogens was promoted resulting in enhanced methane production [73]. The highest specific methane production of 67.91 NL/kg VS was achieved by co-digestion of BSG. This was a significant increase from sewage sludge mono-digestion which produced 13.45 NL/kg VS. Molasses (M), dairy waste (DT) and animal manure (AM), fresh produce (FP) also resulted in substantially enhanced methane production. This indicates the presence of synergistic effects on biogas production from co-digestion M-NW, AM-NW, DW-NW & FP-NW with both CoT & NW municipal sewage sludge. These findings are in line with those of earlier research that has found a significant increase in methane generation when sewage sludge is co-digested with carbs and protein-rich co-substrates [74], [75].

“The carbon-to-nitrogen ratio (C/N) was slightly increased and moved toward the optimum C/N ratios of 20–30 when co-substrates were mixed with sludge in the AD [76]. The increase in the C/N ratio might have led to the observed gas production synergy. Furthermore, the biodegradable percentage peaked, showing that co-digestion enhanced biomass biodegradation in the mix. The increased variety and improved enzymatic machinery of the AD microbial population explain these findings. In the complex substrates associated with the combination of co-substrates and sludge mixture, the microbial community thrives. Hence, this demonstrates a strong connection between the substrate composition, matrix complexity, C/N ratio, and microbial community structure.”.

4. Conclusions

This study focuses on improving the anaerobic digestion process of sewage sludge by co-digesting with biowastes, six organic waste materials as possible co-substrates for enhancement of bio-methane potential and possible impact on digested sludge content in terms of TS, VS, and COD.

- Physicochemical characterization was conducted to obtain the composition of the anaerobic digestion feedstock was carried out before anaerobic digestion. It was found that parameters such as TS, VS, COD, C/N ratio and TA can boost or hinder biogas production.

- The enhancement of biogas was carried out and the results presented show that both NW and CoT sewage sludge contains a high biochemical methane potential.
- However, when compared to sludge mono-digestion, co-substrates increased bio-methane yield by three to six times. Although high solid content co-substrates generated significantly more methane gas, they also increased the risk of organic overloading and digested sludge instabilities.
- Co-substrates such as molasses, food waste, animal manure, brewer spent grain and fresh produce waste performed well at 25% co-digestion ratios.
- Furthermore, except manure, all other high solid content co-substrates resulted in increased VS and COD residuals in the digestate material.”
- According to the findings of the study, anaerobic co-digestion of sludge and BSG has a 90.25 percent efficiency in COD removal and a 67.27 percent efficiency in total solids removal.
- Based on the presented result, anaerobic co-digestion is a cost-effective approach that could help WWTPs become more energy self-sufficient and waste management become more sustainable.””

CRedit authorship contribution statement

Khuthadzo Mudzanani: Conceptualization, Methodology, Validation, Data curation, Writing – original draft. **Sunny E. Iyuke:** Supervision, Conceptualization, Writing – review & editing. **Michael Daramola:** Supervision, Conceptualization, Formal analysis, Methodology, Investigation, Visualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Further reading

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