Semi-Continuous Testing: The Effect of Mechanical Pre-treatment on Degradation of Complex Organic Matter

Ebaye S. Bejor[a] *, Samson Konebimotu[b], Emmanuel Ekeng Ewa[b]

Department of Civil Engineering, Faculty of Engineering, Cross River University of Technology Calabar, Nigeria.

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Abstract: This study investigates the impact of pre-treatment intensity on biogas production in anaerobic digestion processes using three different substrates: BPWSB, TWSB, and PWSB. The experimental design includes semi-continuous tests with various pre-treatment levels (PTLs) to analyze specific methane yields and process stability indicators. Reactor performance comparisons reveal differences in methane production profiles among substrates, with GWSB generally outperforming BPWSB and PWSB. Additionally, PTL4 consistently yields higher specific methane yields than PTL3 and PTL2 across all substrates due to its greater surface area. The specific methane yields for BPWSB, TWSB, and PWSB are comparable but not identical. The highest specific methane yield was produced by BPWSB for PTL2 (M=225.6, SD=57.7), while the lowest was produced by GWSB (M=181.2, SD=51.9). The results suggest that the specific methane yields for BPWSB, TWSB, and PWSB are similar but not identical. Again, BPWSB PTL3 (M=255, SD=63.5) differed significantly from the others, whereas GWSB had the lowest specific methane yield (M=211.7, SD=56.6). Likewise, BPWSB PTL4 outperformed the other substrates (M=290.3, SD=69.3), while GWSB PTL4 had the least (M=218.3, SD=69.4). Intensive pre-treatment shows no detrimental effects on system stability, with reduced foaming tendency observed at higher pre-treatment intensities. These results indicate that carefully choosing pre-treatment methods can boost methane yields while addressing operational difficulties, emphasizing the significance of pre-treatment in sustainable waste management and renewable energy generation. Future studies could investigate further pre-treatment methods and their long-term impacts on biogas output and system stability, offering more comprehensive insights into process refinement.

Keywords: Pre-treatment, Methane yields, Operational challenges, Sustainable waste management, Renewable energy production, Anaerobic digestion, Biogas production, System stability, Process optimization, Substrate-dependent responses

1. Introduction

Energy is vital for economic development, but many developing nations, like Nigeria, struggle with energy access and reliability. Nigeria faces challenges such as frequent power interruptions, reliance on expensive self-generated energy, and limited access to clean energy, particularly in rural areas. These issues deter economic growth and aggravate poverty. Improving the energy situation requires targeted interventions and investments in renewable energy technologies.

Biogas technology offers developing nations a path to cleaner energy access by generating methane and producing valuable soil conditioners [1]. With its small-scale and low-capital requirements, anaerobic digestion (AD) systems can be decentralized and installed in various locations, including remote areas. This technology has the potential to address energy challenges in countries like Nigeria. Therefore, it's crucial to enhance understanding of AD processes, focusing on mechanical pre-treatment of waste biomass, as explored in this study.

In the field of waste management and renewable energy generation, anaerobic digestion (AD) represents a promising approach for transforming organic substrates into biogas. However, the effectiveness of this process relies on various factors, such as the composition of the substrate and the effectiveness of pre-treatment techniques.

In this study, semi-continuous testing was conducted with a hydraulic retention time (HRT) of twenty days, focusing on the impact of mechanical pre-treatment on the degradation of complex organic matter [2]. Three

substrates: grass waste substrate biomass (GWSB), banana peel waste substrate biomass (BPWSB), and paper waste substrate biomass (PWSB) were subjected to varying levels of pre-treatment (PTLs). The substrates were characterized to ensure uniformity and consistency throughout the experimentation process.

The semi-continuous testing involved feeding the reactor once a day, following a carefully designed feeding regime custom-made to each substrate's pre-treatment level. The testing period spanned 60 days, during which methane production was monitored as a key indicator of anaerobic digestion efficiency [3]. This duration was chosen to align with desirable cost reductions and process optimization strategies while ensuring comprehensive data collection.

The experiment using Automatic Methane Potential Test System (AMPTS II) and Bioreactor Simulator (BRS) bioprocess for batch testing and semi-continuous testing was performed in the AD lab at the University of Sheffield. The study's aim is to investigate the effects of various mechanical pre-treatment methods on the size reduction of different organic materials and their potential to degrade under anaerobic conditions.

The study focuses on semi-continuous testing to assess the impact of mechanical pre-treatment on the degradation of complex organic matter. To investigate the difference in methane production at the different physical pre-treatment levels. Through this investigation, the research seeks to establish a relationship between pre-treatment processes and the effectiveness of organic matter breakdown. Also, the study aims to utilize semi-continuous testing as a means to optimize mechanical pre-treatment methods for enhancing the degradation of organic matter.

2. Literature review

2.1. Factors influencing anaerobic digestion process and biogas production

According to Rivard et al. [3], polymeric substrates require a digestion time of 60 to 90 days to be fully digested. The effect of particle size distribution on the operation and optimization of the AD process on the different pre-treatment levels and other relevant process parameters on anaerobic digestion of substrate waste biomass in the gas yield were also studied. The effect of hydraulic retention time (HRT) on the anaerobic digestion of wheat straw was also noted by [2]. A 20-day HRT for the anaerobic digestion of maize was also reported by [4]. When the HRT is less than 2 days, the anaerobic sequential batch reactor treating a dilute waste stream did fail because the HRT was too short to allow for microorganism growth to exceed the limits [5]. Previous research has shown that changing the feeding sequence can increase operational stability while also changing the diversity, dynamism, and evenness of the microbial communities [6]. Studies by Lemmer and [7] - [9] have explored how changes in feed affected the biogas production rate in terms of rise and fall of biogas over time.

2.2. Influence of particle size on biogas production and anaerobic digestion efficiency

Several studies have shown that when particle size increases, the surface area exposed to the bacteria to produce biogas reduces [10], [11], [12]. Studies have indicated that smaller particles play a greater role in biodegradation due to their larger specific surface area compared to larger particles. This conclusion stems from the understanding that the biodegradation and hydrolysis of substrate biomass primarily occur on the surface, where hydrolytic anaerobic microbial organisms, supported by extracellular enzymes, are predominantly attached [12], [13], [14], [10], [15], [16], [17]–[19]. Biogas methane content in a reactor is affected by the kind of substrate used and how efficiently each stage of the anaerobic digestion process is working in that reactor under steady - state conditions [20].

2.3. Optimal pH conditions in anaerobic digestion systems

The optimum pH range in an anaerobic digester is 6.8 to 7.2 in the anaerobic digestion process. However, a range of 6.5 to 8.0 may be tolerated by the digestion process [21], [22]. According to literature data [18] the IA:PA ratio increasing from 0.2 to 0.4 suggests a steady mode of operation for the reactor but rising over 0.4 indicates an unstable digester.

2.4. Effect of particle size on carbon bioavailability and hydrolysis in anaerobic digestion

The organic material and increased unit surface area exposed to enzyme attack may have contributed to this by improving carbon bioavailability and hydrolysis of the treated substrate. Additionally, according to [23], [24]–[26], the particle size of a substrate may have an influence on the efficiency of biological processes like anaerobic digestion (AD). Larger surface area is exposed to enzyme attack on smaller particles per unit of time, which could boost the processed material's carbon accessibility and hydrolysis [27], [23], [28], [29]. Drawing upon findings from prior research, the researchers uncovered the particle size paradox. Their investigation revealed that factors beyond just the average size are crucial, as the rate of gas production per unit surface area diminishes significantly with the reduction in particle size, particularly for smaller particles. Similarly, smaller particle size results in a higher unit surface area that is exposed to enzymatic attack, which could also boost carbon availability and hydrolysis of the mechanical treated material [27], [30], [28], [29].

2.5. Mitigating foaming tendencies through enhanced pre-treatment intensity

Lignin is considered as the most recalcitrant to biological deconstruction due to its irregular, complex, and highly heterogeneous aromatic structure [31]. Elevating the intensity of pre-treatment is anticipated to mitigate foaming tendencies. This assertion stems from the findings of numerous scientific inquiries [32], [33], [14], [34], which have highlighted organic overloading as a potential cause of foaming in digesters. This occurs due to the surplus compounds remaining undegraded by the bacteria within the digesters, potentially fostering the accumulation of hydrophobic or surface-active by-products conducive to foam formation.

The remaining sections of this paper are organized as follows: Section 3 provides an overview of the experimental design and describes the materials and methods employed in this investigation. Section 4 offers an analysis of the experimental findings. Section 5 presents the conclusions drawn from this study and proposes potential avenues for future research.

3. Materials and methods

3.1. Feedstock and inoculum

Four types of waste biomass were utilized in this study: (i) Paper waste sourced from the University of Sheffield Energy Group Offices, (ii) Banana peels collected from households, (iii) Grass obtained from the University of Sheffield, and (iv) Tomato waste procured from the Moor Market in Sheffield. The feedstock was separated from three distinct routes. Once a sufficient sample (20kg) was amassed, the raw waste underwent screening to eliminate any contaminants, if present, and was subsequently homogenized for characterization and further size reduction. Additionally, anaerobic fresh active digestate was gathered from the existing mesophilic AD energy plant at Blackburn Meadows (BbM) wastewater treatment works (WwTW). Prior to utilization, the fresh active digestate from mesophilic digesters underwent filtration using a 1mm mesh sieve to eliminate solid materials for batch or semi-continuous testing.

3.2. Feedstock preparation and mixing

3.2.1. Feedstock particle size comminution

Using an analytical weighing balance, an amount of each waste feedstock was measured and divided into four equal portions. Each type of biomass underwent four particle size reduction methods, which commenced with a coarse chopping/shredding process (PTL1), followed by a finer chopping process (PTL2), and finally a maceration/mincing process (PTL3). The process is detailed in table 1, illustrating the nomenclature for each biomass size reduction, along with variations in pre-treatment levels, processing times, and reduction mechanisms. Notably, pre-treatment 2 (PTL2) entailed passing 3/4 of the waste through a Mincer Ring RAUT 12 16# for 2 minutes, while pre-treatment level 3 (PTL3) involved passing 2/3 parts of the waste through a food processor with a cut 5200 (Grinder) for 3 minutes. Pre-treatment level 4 (PTL4) included passing the third part of the feedstock through a Mincer Ring #12 6mm and then through a Grinder (5200) for 5 minutes, respectively. The feedstock was stored at 50°C prior to the experiment. Each fraction was characterized by its particle size distribution using the

most pertinent method. However, the production of biogas yield varied depending on the type of feedstock employed.

Table 1. Nomenclature of the pre-treatment of each biomass

				Pre-treatment (PT) level					
Biomass Type	Units	Quantity	Water ($H2^0$) addition (g)	1	2		3		4
Paper waste	kg	5	3	Shredded	PW1 -3 passed through a mincer for 2mins	Divided into three equal parts	Part 2 -3 PW2 slurried with water and 3min in food processor	Divided into two equal parts	Part 3 PW3 slurried with water and 5min in food processor and in mincer
Banana peel	kg	8	-	Manual chopping	BPW1-3 passed through a mincer for 2mins	Divided into three equal parts	Part 2 -3 BPW3 in food processor 3minutes	Divided into two equal parts	Part 3 BPW3 slurried with water and 5min in food processor and in mincer
Grass waste	kg	6	-	As collected	GW1-3 passed through a mincer for 2mins	Divided into three equal parts	Part 2 -3 GW2 slurred with water and 3 min in food processor	Divided into two equal parts	Part 3 GW3 slurried with water and 5min in food processor and in mincer
Tomato waste	kg	6	-	As collected	TW1-3 passed through a mincer for 2mins	Divided into three equal parts	Part 2 -3 TW2 slurred with water and 3 min in food processor	Divided into two equal parts	Part 3 TW3 slurried with water and 5min in food processor and in mincer

3.3. Analytical parameters measured for substrate digestion and digestate

Table 2 presents the parameters investigated in this study concerning the physicochemical and biological composition of the feedstock, including total solids (TS) and volatile solids (VS), pH, alkalinity, elemental analysis, biogas composition, and volume. The content of total solids (TS) and volatile solids (VS) in the liquid digestate

from the digesters was assessed. pH and alkalinity analyses were conducted to evaluate the stability of the digestate. Methane and CO_2 levels in the biogas were quantified. Reagents for the study were procured from Fisher Scientific (Loughborough, United Kingdom), with chemicals graded on a laboratory scale unless otherwise specified.

Table 2. Analysed experimental testing parameters

Parameter	Substrate	Methane	Digestate
PS	○	-	-
TS/VS	○	-	○
CHNS	○	-	-
BMP	○	-	-
pH	-	-	○
Alkalinity (PA, IA, TA)	-	-	○
Biogas composition	-	○	-
Methane volume	-	○	-

○ Measure
- Not measure

3.4. Preparation of the reagents and indicator

CO_2 - fixation: 3 mol of NaOH solution was prepared by dissolving 240g of the substance in 1.5 litres of distilled water and making the solution to 2 litres using distilled water. The experiment was performed in a fume cupboard due to the heat generated. 10ml of 0.4% Thymolphthalein-pH indicator was mixed with 2 litres of the 3 mol NaOH solutions. 80ml of the mixture containing NaOH solution and Thymolphthalein pH indicator was transferred to each of fifteen 100ml glass bottles.

3.4.1. pH

The pH of the sample's biomass is measured using a pH probe meter Omega PHH-37 with Omega PHE 1335 probe. Before the use of the pH meter, Buffer solutions used for calibration were (pH 4.01, 7.00 and 10.1). Deionised H_2O was poured into two beakers of about 200ml each and this was used to rinse the pH probe. Equally the beakers were emptied and refilled for a rinse of the probe meter. This was done during the time of measurements and at the end of the measurement. The measurement of the pH was taken immediately the biomass samples are taken out of the reactor to avoid the samples volatiles being evaporated or the evolution of dissolved CO_2, thereby, keeping the reading accurate without alteration. During the pH measurement substrate, biomass samples were well stirred to ensure the samples are properly homogenized before the pH measurement. The pH

meter accuracy was ± 0.03 and a resolution of 0.01, but according to the standard method of water and wastewater 4500-H+ [19] on the normal basis, the accuracy of the PH meter is ± 0.1 pH.

3.4.2. Preparation, determination of total solid TS and volatile solid VS

After the sample had been properly homogenised, the anaerobic fresh active digestate and substrate are assessed for total solid (TS) and volatile solids (VS). The fresh active digestate is poured into a crucible, while a portion of the well-mixed biomass sample is transferred to weighed empty crucibles using a spatula. The weight of the wet samples plus the empty crucible weight is recorded. The biomass sample is dried in an oven at 105°C for 24 hours and then weighed to the nearest sensitivity of 0.1mg after cooling in a desiccator. Subsequently, the biomass samples are transferred to a box furnace heated at 550°C for two hours, then weighed again after cooling to room temperature. Standard methods 2540G are employed for measurement, with units in grams (g) [19]. After each set of samples, crucibles are washed with detergent and rinsed with deionized water before further analysis. Total solids (TS) and volatile solids (VS) are determined using equations 3.1 - 3.3.

$$\% \text{ Total solid (TS} = \frac{w_3 - w_1}{w_2 - w_1} \quad (3.1)$$

$$\% \text{ Volatile solids (VS)} = \frac{w_3 - w_4}{w_2 - w_1} \quad (3.2)$$

$$\% \text{ (VS based on total solids)} = \frac{w_3 - w_4}{w_3 - w_1} \quad (3.3)$$

Where:
W_1 is the empty weight of the crucible measured in (g).
W_2 is the measured weight of the crucible with a fresh active digestate, or substrate measured in (g).
W_3 is the substrate or digestate sample weight after drying in an oven at 105^0C measured in (g).
W_4 is the measured weight of the crucible and a wet sample weight after the heating at 550^0C measured in (g).

3.4.3. The alkalinity of the biomass sample

The alkalinity of liquid digestate samples was determined using Standard Method 2320 B [19]. Before analysis, the digestate sample was sieved for homogeneity. Then, 5ml of liquid digestate was mixed with 50ml of deionized water. The pH of the sample was measured using a 0.25N sulphuric acid solution and a pH probe with magnetic stirring to prevent fouling. To prevent cross-contamination, the pH probe was calibrated with buffer solution at the start of titration and rinsed with deionized water between measurements. Three alkalinity ratio measures (PA, IA, and TA) were analyzed based on initial pH and pH endpoints, as per [35], outlined in table 3. The liquid digestate sample was titrated as mg CaCO3l-1 using an automatic digital S1 analytics titroline 5000 titrator.

Table 3. Alkalinity Definition [36]

Type of Alkalinity	Definition	Initial pH	Endpoint pH
PA	buffer of bicarbonate	pH of sample	5.7
IA	Buffer of Volatile fatty acid (VFA)	5.7	4.3
TA		pH of sample	4

Alkalinity was calculated according to mg $CaCO_3l^{-1}$:

$$Partial\ Akalinity\ (PA) = \frac{A_{5.7} \times N \times 50000}{V_{substrate}} \quad (3.4)$$

$$Total\ Alkalinity\ (TA) = \frac{(V_{4.0} \times V_{4.3} \times V_{5.7}) \times N \times 50000}{V_{substrate}} \quad (3.5)$$

$$Intermediate\ Akalinity\ (IA) = \frac{B_{5.7} \times N \times 50000}{V_{substrate}} \quad (3.6)$$

Where:

A represent the volume of H_2SO_4 added in mL to attain the end point Intermediate endpoint pH 5.7.
B represent the volume of H2SO_4 added in mL to attain the ultimate endpoint pH 4.3.
N is the titrant's normality, H_2SO_4.
V represents the sample volume in ml.
From equation 3.4 to 3.6, it indicates the titrant volume used to the endpoint point of analysis is 4.0, 4.3 and 5.7 ml respectively.

3.5. Estimation of theoretical maximum methane production

The composition of substrate biomass, including carbohydrates, proteins, and lipids, influences the methane and carbon dioxide content in biogas, thereby affecting energy production [37]. Optimal methane production demands a carbon-to-nitrogen ratio of 25:1. The Buswell equation [38] enables the calculation of water usage and methane and carbon dioxide production when a known mass of volatile solid (VS) undergoes anaerobic digestion. [38]:

$$C_C H_h O_O N_O S_s + \frac{1}{4}(4c - h + 2o + 3n + 2s)\ H_2\ o \rightarrow \frac{1}{8}(4c - h + 2o + 3n + 2s)CO_2 + \frac{1}{8}(4c + h - 2o - 3n - 2s)CH_4 + nNH_3 + sH_2S \quad (3.7)$$

Various methods exist for calculating the calorific value (CV) of biomass or solid waste, including consideration of their physical composition, proximate analysis, or ultimate analysis involving elemental content (C, N, H, S, O) [39] - [41]. Studies have shown that determining the CV based on elemental composition or ultimate analysis yields the most accurate and precise results [41], [42]. Thus, in this study, the theoretical CV of four substrate biomass samples (BPWSB, GWSB, PWSB, and TWSB) was determined using Dulong equation 3.8 and 3.9, enabling the calculation of potential energy content from anaerobic digestion of these biomass samples.

$$\mathbf{HHV} = (337C + 1419(H - 1419\frac{O}{8}) + 93S + 23.26N \quad \mathbf{(3.8)}$$

$$TCV = (34.1C + 102H + 6.3N + 19.1S - 9.85O)/100 \quad \mathbf{(3.9)}$$

3.6. Composition of elements (CHNS)

A sample that had been weighed (1.8 - 2.2 mg) and crushed to remove air inclusions was sealed in tin foil. The Vario Micro Cube's CHNS analysis mode was used. The results were corrected for blanks. A daily factor correction is provided by running sulphanilamide standards (x3) every 12 samples.

3.7. Experimental procedure

3.7.1. Sample preparation and anaerobic condition employed for BMP testing and methane production

The samples were kept in a freezer at 4^{0}C. Prior to starting the BMP tests, the fully automated methane potential test system (AMPTS II) and software were configured as described in the bioprocess manual. Table 4 provides a description of the digesters that were used for the semi-continuous test. A 3M NaOH solution was prepared in the fume cupboard. The chemical mixtures (3M NaOH and pH indicator thymolphthalein) were carried out in accordance with the manufacturer's instructions, taking all necessary precautions. Table 5 shows the batch test conditions used in this study to promote degradation/ultimate rate of methane (CH_4) production and characteristic kinetics during anaerobic material preparation.

Table 4. Summary of the experimental methodology of the continuous stirred tank reactor (CSTR)

	SET1(R1+2)	SET1(R3+4)	SET1(R5+6)
PT Levels	PT4	PT4	PT4
Substrate	GWSB	BPWSB	PWSB
	SET2(R1+2)	SET2(R3+4)	SET2(R5+6)
PT Levels	PT3	PT3	PT3
Substrate	GWSB	BPWSB	PWSB
	SET2(R1+2)	SET2(R3+4)	SET2(R5+6)
PT Levels	PT2	PT2	PT2
Substrate	GWSB	BPWSB	PWSB
Reactors numbers	6 (2 duplicates)		
Feed	Wet Substrate waste biomass (WSWB)		
Organic loading rate (OLR)	3gVS wet/day		
Reactor size and size	2000ml CSTR		
Allowed headspace	300ml		
HRT	20 each PT level		
Fed per day	1 time daily		
Interval of feed	24hh:mm		
Mixing	Mechanical stirring		
Inoculum	Fresh active digestate		
Reactor temperature	38°C		

Operation of feed	Semi -continuous (Manual)

Table 5. Batch testing employed condition

Conditions Employ BMP Testing	Freshly Active Digestate (Inoculum)	To Maintain Active Anaerobic Bacteria and Promote CH_4 Production Rate
	Mesophilic Conditions 38°C	For high methanogenic microbial activity
	Short hydraulic retention time	Average of 15 to 30days is required to treat waste
	Mechanical stirring	To ensure a very good mixture of the inoculum and biomass substrate
	Large inoculum to substrate ratio 3:1 (VS basis)	To enhance the methane (CH_4) production rate
	Automated incubation unit 15 x0.5 with the headspace of 100ml	15 incubation units are analysed the same time

3.7.2. Leak test

A leak test was performed for each of the reactors by creating some overpressure. This was done by blocking one of the metal tubing ports and the air was injected through the remaining port and the reactor was immersed in water and monitored if any air bubbles would escape from the reactors. The Thermostatic water bath was switched on and set at 38°C. The gas volume measuring device was flushed with methane calibration gas at 5l/min for 60 seconds to create the anaerobic condition.

3.8. Batch testing set-up and monitoring

The results obtained provide insights into the influence of particle size distribution on the kinetics of the anaerobic digestion (AD) process and the overall biodegradability and methane potential of the system. These findings enable recommendations regarding the optimal pre-treatment level. The experiment involved conducting Biomethane Potential (BMP) tests on biomass samples characterized for particle size distribution. Using BMP equipment, each substrate underwent testing in triplicate to ensure statistical robustness. An anaerobic digestate inoculum was obtained and filtered for homogeneity before being distributed into test bottles. Each bottle received a measured amount of substrate and inoculum, maintaining a specific inoculum to substrate ratio to optimize methane production and prevent digester failure. Blank and control samples were also prepared for reference. The bottles were sealed and placed in an incubation unit set at 38°C. Methane production was monitored using data logging software (AMPTS II), while the CO_2 absorption unit facilitated accurate methane measurement. The accumulated methane volume was recorded post-digestion, and the biochemical methane potential was calculated accordingly using equation 3.10.

$$BMP = \frac{V_{substrate} + Inoculum - V_{innoculum}}{Substrate\ \ gram\ VS\ added} \qquad (3.10)$$

Where:

BMP = Biochemical methane potential is the normalized volume produced per gram VS of substrate added (Nml/gVS).

3.9. Semi-continuous testing

The experiment aims to determine the maximum biogas production rate and assess the effects of various pre-treatment methods on stability, production kinetics, and other factors. Different levels of pre-treatment are applied to banana peel, grass waste, and paper waste, which are digested in R1-R6 digesters seeded with fresh active digestate. Each reactor, housed in a 2000ml bottle with a headspace of about 300ml, is equipped with ports for substrate feeding, discharge of digested sludge, and pH and temperature measurement. The system creates an anaerobic environment using sealed off inlet and outlet funnels and continuously records biogas production using bioreactor software. Digestate removal before substrate feeding reduces the risk of foam clogging gas tubes, while feeding through a hydraulically sealed inlet minimizes air entry to the reactor headspace, optimizing methane production.

3.9.1. Gas volumes for BMP assay and semi continuous testing

The volume of the gas was gauged using an ultra-low flow gas flowmeter (Flow, Bioprocess Control, Sweden). The flowmeter's precision was 1%, and its resolution was 10 mL ±1 mL. The processing unit of the flowmeter used the flowmeter cell volume calibration value. The flowmeter adjusted the gas measurements automatically to 0^0 C and 1 atm (STP).

4. Results and discussion

The study conducted semi-continuous testing with a hydraulic retention time (HRT) of twenty days for various pre-treatment levels of substrate biomass. Three substrates, categorized into different pre-treatment levels (PTLs), were tested: grass waste (GWSB), banana peel waste (BPWSB), and paper waste (PWSB). Each substrate underwent pre-treatment levels 2-4. The experiment spanned 60 days to assess methane production variability, a duration chosen to align with desirable cost reduction and process efficiency optimization associated with shorter HRTs. Materials are easily mechanically broken down while some are not (PWSB and GWSB). The Calculated organic loading rates (OLRs) for the three substrates with water addition are shown in tables 6 to 8. However, the bioprocess laboratory-scale bioreactor was fed on a daily average value for 7-day week with an organic loading rate in wet 3gVS/day and the parameters measured during the semi-continuous testing is shown in table 9.

Table 6. Calculated organic loading rate (OLR) of banana peel waste substate biomass

Substrate Biomass (BPWSB)		
PTL2	PTL3	PTL4
Mass in	Mass in	Mass in
Reactor volume (RV) =1.7L		
Organic loading Reactor (OLR) 3kgVS/m3day		
Feed (f) :3*1.7=5.1		
Feed VS (FVS): 9.090=0.0909		
F/FVS: 56.11 g wet/day		
What about 5 days a week: 2*7/5=2.8		
Digester feed per day: 56.11+ 2.8 = 58.91 g wet/day		
Hydraulic retention Time (HRT): 28.86		
With water addition = Amount of water/day (ml) = 26.094		
Final HRT:RV/ (feed per day +H20)/1000 = 20		

Table 7. Calculated organic loading rate (OLR) of grass peel waste substate biomass

Substrate Biomass (GWSB)		
PTL2	PTL3	PTL4
Mass in	Mass in	Mass in
Reactor volume (RV) =1.7L		
Organic loading Reactor (OLR) 3kgVS/m3day		
Feed (f) :3*1.7=5.1		
Feed VS (FVS): 20.33=0.2033		
F/FVS: 27.81 g wet/day		
What about 5 days a week: 2*7/5=2.8		
Digester feed per day:27.81 + 2.8 =30.61g wet/day		
Hydraulic retention Time (HRT): 55.5		
With water addition = Amount of water/day (ml) = 54.39		
Final HRT: RV/ (feed per day +H20)/1000 = 20		

Table 8. Calculated organic loading rate (OLR) of paper waste substate biomass

Substrate Biomass (PWSB)		
PTL2	PTL3	PTL4
Mass in	Mass in	Mass in
Reactor volume (RV) =1.7L		
Organic loading Reactor (OLR) 3kgVS/m3day		
Feed (f) :3*1.7=5.1		
Feed VS (FVS): 20.33=0.2033		
F/FVS: 25.09 g wet/day		
What about 5 days a week: 2*7/5=2.8		
Digester feed per day:25.09 + 2.8 =27.89g wet/day		
Hydraulic retention Time (HRT): 60.96		
With water addition = Amount of water/day (ml) = 57.113		
Final HRT: RV/ (feed per day +H20)/1000 = 20		

Table 9. Parameters measured during semi-continuous testing

Physical And Chemical Parameters	Frequency	Reference
PH	Twice each week	[218]

Total Alkalinity (TA), Partial Alkalinity (PA)And Intermediate Alkalinity (IA)	Twice each week	2320B [218]
Total Solid (Ts) And Volatile Solid (VS)		[205]
Anaerobic Biogas Flow	Daily	Automatic Bioprocess
Biogas Composition	Daily	Automatic Bioprocess
Volatile Solid Destruction Measure (VSD)	Twice each week	

All digesters were initially inoculated and subjected to batch mode digestion until the stability of biogas production. The daily loading rate started with pre-treatment level 4 in the first 20 days. This was done for the anaerobic microbial organisms to feed on the substrate and improve the anaerobic degradability. After the first 20 days, the digester is fed with pre-treatment level 3 followed by pre-treatment level 2. Before feeding the freshly anaerobic substrate biomass for each substrate, an equal amount of digestate was withdrawn daily. This was done to maintain the recommendation headspace (300ml) by bioprocess and to avoid failure of the AD system while allowing the biomass substrate to have a vigorous mixture to promote the activity of a microbial organism in the bioreactors, to enhance the rate of methane production as well maintaining a constant volume of the bioreactor. The bioreactor system is well built with mechanical stirrers, and this presents an opportunity also for acclimatization of the freshly filtered digestate in the same vessel. The bioreactors were run in duplicate as shown in table 10.

Table 10. Duplicate reactor used for semi-continuous testing of three substrate biomass degradation

Bioreactors	Days of Feeding			Substrate Biomass	Feeding Sequence
	(1-20d)	(21-40d)	(41-58d)		
R1	PT4	PT3	PT2	GWSB	PT4 PT3 and PT2
R2	PT4	PT3	PT2	GWSB	PT4 PT3 and PT2
R3	PT4	PT3	PT2	BPWSB	PT4 PT3 and PT2
R4	PT4	PT3	PT2	BPWSB	PT4 PT3 and PT2
R5	PT4	PT3	PT2	PWSB	PT4 PT3 and PT2
R6	PT4	PT3	PT2	PWSB	PT4 PT3 and PT2

4.1. Grass waste substrate biomass

4.1.1. Biogas output methane production of the GWSB

The results indicate that a temperature shock occurred in the semi-continuous test incubation digesters (R1-R2) around day 22/23, leading to a significant decrease in methane content in the biogas. This phenomenon could be attributed to the nature of the substrates and feeding patterns. However, methane content gradually increased after system recovery, with fluctuations observed as particle size (PS) decreased over time. Figure 1 illustrates the percentage (%) and daily average methane (CH_4) production from semi-continuous testing of GWSB, while

Appendix A1 presents experimental results, showing an average daily biogas methane flow rate of approximately 1149 ml/day over 9 weeks. Figure 2 depicts the impact of hydraulic retention time (HRT) on biogas production. The methane content peaked on day 8 (68.5% for R1) and day 3 (67.4% for R2), with minimum levels recorded on day 23 (25.5% for R1) and day 23 (30.3% for R2). Notably, methane output remained relatively stable over time, with R2 displaying greater stability than R1. Additionally, PTL4, processed for five minutes in a mincer and grinder, yielded peak biogas methane content for R1 and R2, respectively, possibly due to accelerated breakdown and increased microbial activity. The proportion of biogas methane in samples of grass waste substrate biomass (GWSB) ranged from 55.1% (R1) to 58.9% (R2), aligning with typical methane content in GWSB-derived biogas. Moreover, the effect of HRT on anaerobic digestion of wheat straw was observed [2], with longer HRT associated with higher biogas production and methane content. The present results support [5] 's assertion that when the HRT was less than 2 days, the anaerobic sequential batch reactor treating a dilute waste stream did fail because the HRT was too short to allow for microorganism growth to exceed the limits.

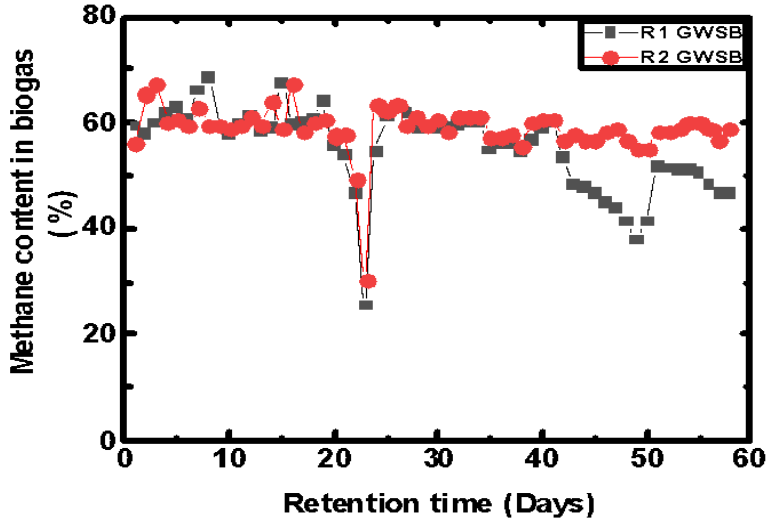


Figure 1. Percentage biogas methane (CH_4) content produced from the degradation of GWSB (R1 and R2)

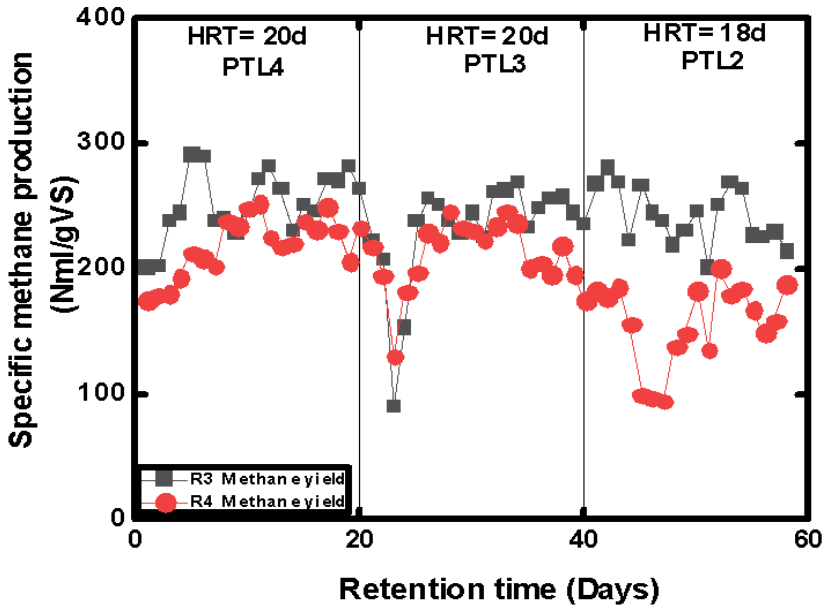


Figure 2. Specific methane (CH_4) production of the degradation of BPWSB (R3 And R4)

4.1.2. The specific production rate of methane (CH_4) in semi-continuous testing of GWSB

The results depicted in figure 3 illustrate the specific methane production trends in the GWSB bioreactors. Initially, both reactors experienced a notable increase in methane yield, reaching 333 Nml/gVS within the first five days, likely attributed to the degradation of readily available organic components in the grass waste. However, methane production subsequently declined in both reactors to around 280 Nml/gVS. The introduction of PTL3 led to a significant drop in methane production, indicating the influence of larger particle size (PS) with lesser surface area, which requires more time to digest and affects biogas output. This suggests that hydraulic retention time (HRT) and temperature shock may have impacted methane production. Notably, methane production was consistently higher in R2 compared to R1, suggesting more efficient digestion processes in R2. However, beyond a certain point, further reduction in particle size did not correspond to increased biogas yield, emphasizing the complex relationship between particle size, HRT, and methane production. The biogas output depicted in figure 4 further reflects the impact of HRT and particle size reduction on methane production, with PTL4 exhibiting the highest methane production rate. Previous research has shown that changing the feeding sequence can increase operational stability while also changing the diversity, dynamism, and evenness of the microbial communities [6]. Studies [7] - [9] have explored how changes in feed affected the biogas production rate in terms of rise and fall of biogas over time.

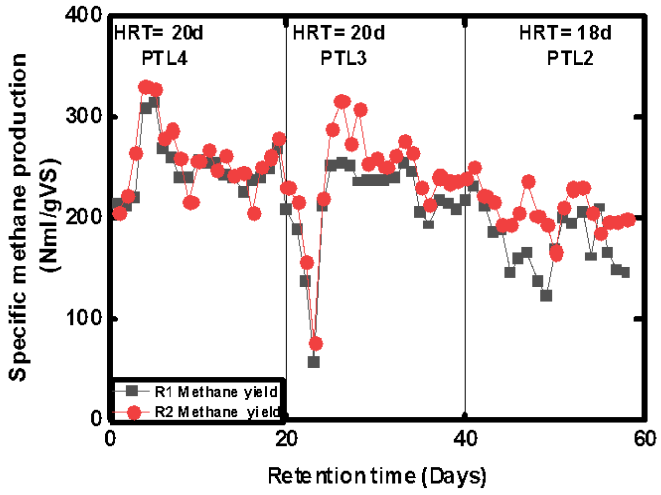


Figure 3. Semi-continuous testing showing specific CH4 production of GWSB (R1 and R2)

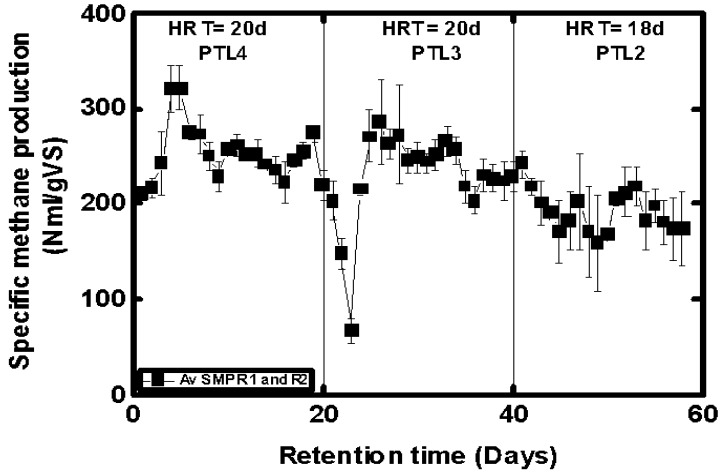


Figure 4. Semi-continuous testing showing average specific CH_4 production from GWSB in a bioreactor R1 and R2)

4.2. Banana peeled waste substrate biomass

4.2.1. Percentage and average methane production rate of BPWSB

The semi-continuous test results of biogas methane content from BPWSB reactors are illustrated as percentages in figure 5. A notable drop in methane content occurred on days 23 for reactors R3 and R4, attributed to temperature shock, likely influenced by substrate composition and feeding order. Daily average methane flow rate over nine weeks was approximately 1102 ml/day. HRT was found to impact biogas methane content, with R3 and R4 producing about 69% and 64% methane, respectively, on earlier days, contrasting sharply with the 31% and 30% on day 23. Particularly, reactors fed with larger particle size (PS) via grinders and mincers showed reduced biogas production across all PTLs tested [10], [11], [12]. Studies suggest that larger particle sizes decrease the surface area available for bacterial action, aligning with the higher methane content in PTL4 digesters processed with mincers and grinders [12], [13], [14], [10], [15], [16], [43]–[46] . This stresses the importance of particle size in biodegradation and highlights surface-related mechanisms in substrate hydrolysis, crucial for anaerobic microbial activity.

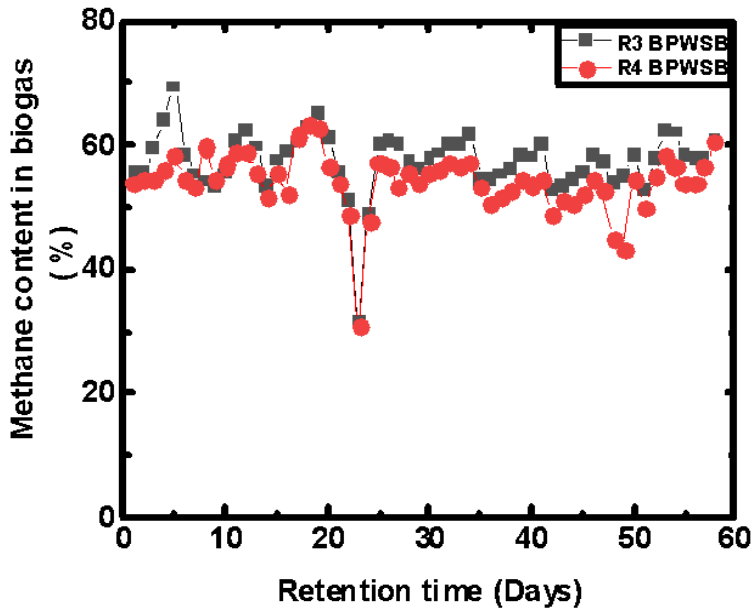


Figure 5. Methane (CH4) content of biogas production of the degradation of BPWSB (R3 and R6)

4.2.2. The Specific Production Rate of Methane (CH_4) in Semi-continuous Testing of BPWSB

Figure 6 depicts the specific methane output for BPWSB reactors R3 and R4, showing similar trends but consistently higher flow rates for R3 compared to R4. Both reactors saw an initial rise in methane production, with R3 peaking at 291 Nml/gVS on day 5 and R4 reaching 252 Nml/gVS on day 11. However, methane flow rates for both dropped significantly by over 50% around day 23, possibly due to changes in system kinetics following PTL3 or destabilization of the AD system. A similar decline occurred for R4 around day 45 after PTL2 feeding. Despite recovery, R3 continued methane production while R4 experienced consistent declines from day 30 to 47, suggesting substrate wash-off. HRT impact on methane production aligns with GWSB findings, supporting the notion that insufficient HRT inhibits microbial multiplication. Both reactors showed sudden declines after each new PTL feed, attributed to feeding sequence and substrate properties. Smaller particles (PTL4) led to methane production peaks, with PTL4 processed for five minutes yielding the highest methane output, followed by PTL3 and then PTL2. The average specific methane production of BPWSB is presented in figure 7 along with standard deviation error bars. PTL4 achieved a specific methane yield of 262 ±21Nml/gVS and has the highest effect on biogas production. With peaks and troughs in methane output, daily PTL4 feeding of the digester promotes the methane production to continually increase. PTL3 achieved roughly 254±12 Nml/gVS, while PTL1 had the lowest methane output at approximately 229±28 Nml/gVS.

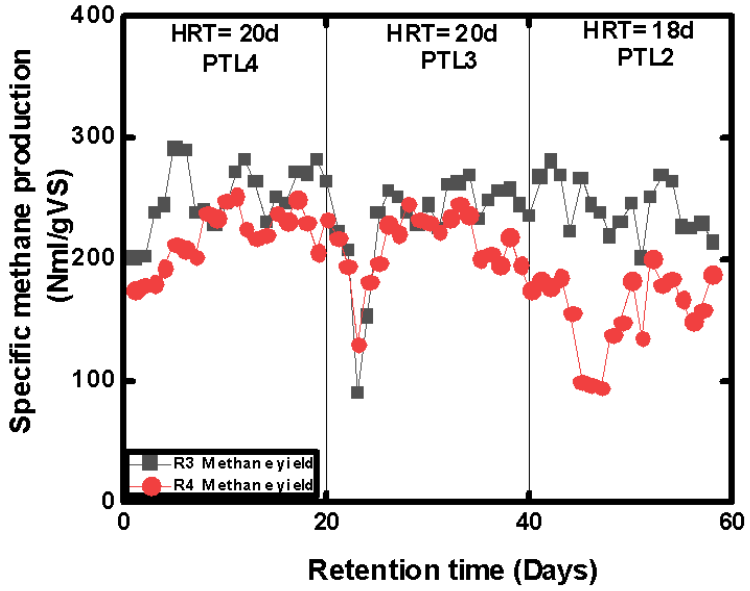


Figure 6. Specific methane (CH4) production of the degradation of BPWSB (R3 And R4)

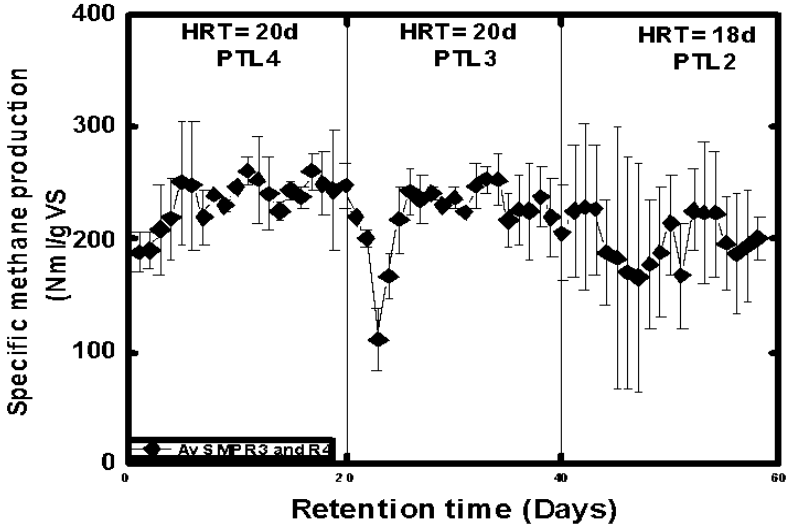


Figure 7. Semi-continuous testing showing average specific CH4 production of BPWSB degradation

4.3. Paper waste substrate biomass

4.3.1. Percentage and average production rate of paper waste substrate biomass

The lab-scale analysis of paper waste substrate biomass, depicted in figure 8, reveals a daily average biogas methane output of approximately 955 ml/day (see Appendix A3). On day 23, a notable temperature shock significantly reduced biogas methane content in reactors R3 and R4, possibly due to substrate composition and feeding order. Both R5 and R6 exhibit similar biogas methane output trends, with R5 showing greater stability. Peaks in methane concentration (CH_4) occurred on days 13 and 27 for R5 and R6, reaching approximately 64% and 66%, respectively, indicating higher output rates for pre-treatment levels 4 and 3. The decline in methane content on day 23 could be attributed to feeding larger particle sizes treated with a grinder (PTL3) and mincer (PTL2), as larger particles require longer breakdown times. HRT likely impacted methane concentration, aligning with findings from other substrate

analyses. Both R5 and R6 show a gradual increase in biogas methane output until the test's end, exhibiting similar patterns to grass and banana peel waste substrate biomass.

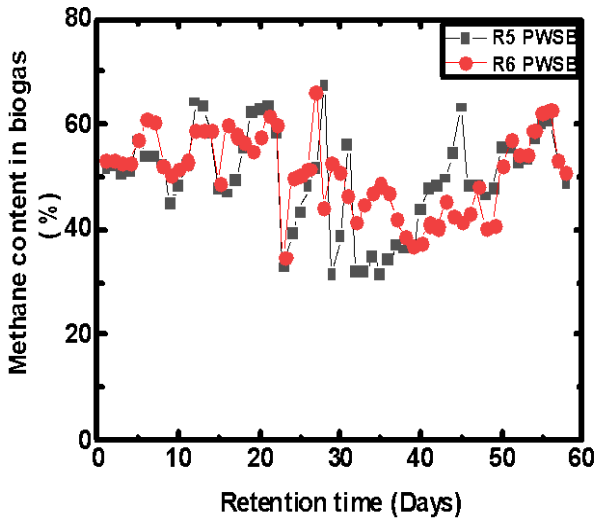


Figure 8. Percentage biogas methane CH4 production of the degradation of PWSB (R5 and R6)

4.3.2. The Specific Production Rate of the Methane (CH_4) in Semi-continuous Testing of PWSB

The specific methane output for paper waste substrate biomass (PWSB) depicted in Figure 9 initially increased across all pre-treatment levels before declining. Particularly, R6 exhibited a higher biogas spike on day 9 compared to R5. PTL4 displayed the highest methane production for both reactors, attributed to its finer particle size distribution and larger surface area for enzymatic hydrolysis. Comparing R6 to R5, R6 consistently produced higher methane output, indicating differences in particle size distribution. The methane production profiles for PTL3 and PTL2 varied, likely due to slight differences in particle size distribution. Both reactors experienced a sudden drop in methane output when fed with PTL3, similar to the response to PTL2 feeding, possibly due to larger particles with lesser surface area. Peaks and troughs in methane output suggest HRT's impact on biogas production rates, as shown in Figure 10. Cumulative methane production, depicted in Figure 11, increased initially with PTL4 feeding but gradually declined over time. Recovery in methane output occurred after a steep decline on day 23, with fluctuations attributed to particle size effects and HRT variations.

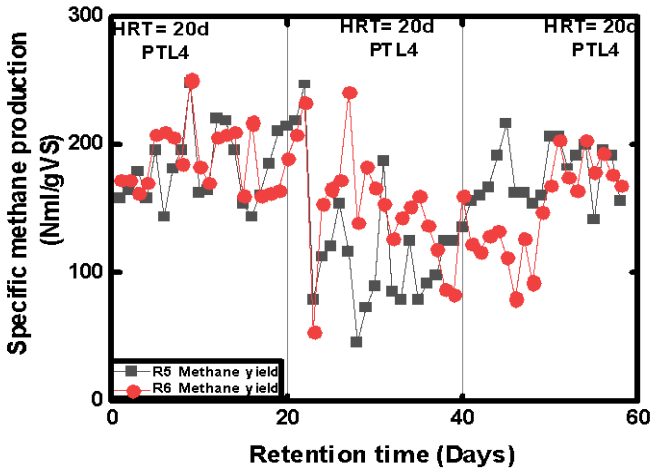


Figure 9. Semi-continuous testing showing specific CH_4 production of the PWSB

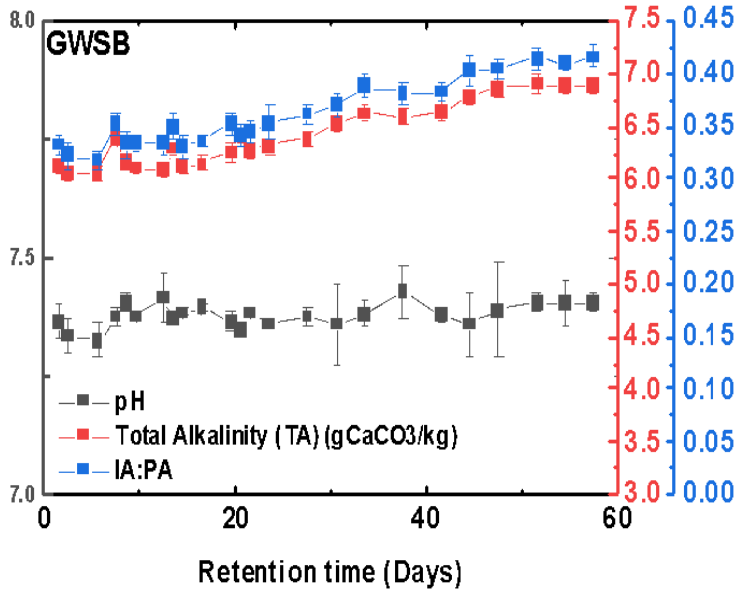


Figure 10. Shows the pH, total and IA:PA alkalinity, for the GWSB digester during the semi-continuous test

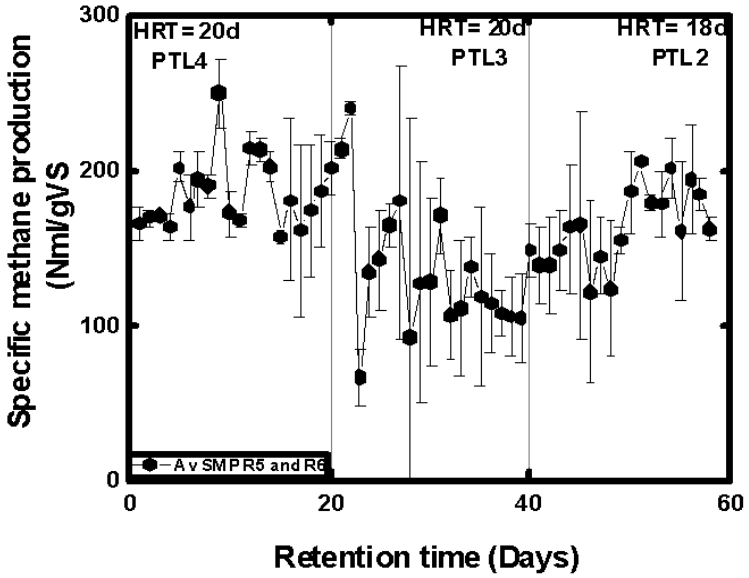


Figure 11. Semi-continuous testing showing average specific CH4 production of PWSB

4.4. Performance of reactor R1 to R6

The biogas methane content within a reactor is influenced by the substrate type and the efficiency of each stage of the anaerobic digestion process under steady-state conditions [47]. In a semi-continuous test with six reactors, duplicates of each sample reactor were used. However, R2, R3, and R6 exhibited higher methane content, indicating more effective digestion activities compared to R1, R4, and R5 with the same substrate. Short hydraulic retention time (HRT) and changes in feed particle size distributions (PTLs) significantly affected reactor performance throughout the test. Notably, PTL4 showed consistent methane production trends for the first 20 days in R1 and R2, while R2 produced more methane with PTL3 and PTL2 thereafter. Similarly, reactor R3 consistently displayed

higher methane output across all PTLs compared to R4. While reactor R5 produced more methane with PTL2 than R6, R6 surpassed R5 in methane production with PTL4 and PTL3. PTL4 generally yielded more methane in the initial 20 days compared to PTL3 and PTL2.

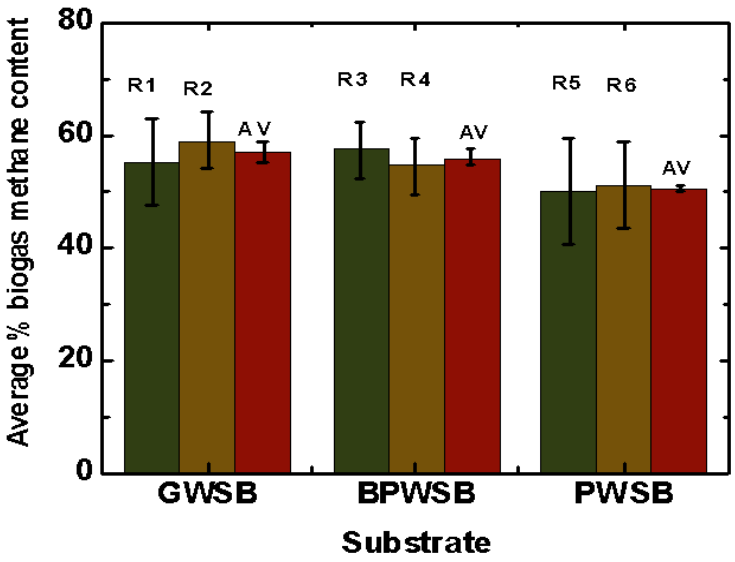


Figure 12. The average % of biogas methane content for GWSB, BPWSB, and PWSB duplicate reactors R1 to R6

Table 12. Average% biogas methane content for reactor R1 to R6 PTLs

	GWSB	BPWSB	PWSB
	PTL4		
R1	61.10±3.02	59.12±4.17	53.92±5.63
R2	60.84±2.97	56.72±3.28	55.62±3.59
AV	60.97±0.13	57.92±1.28	54.77±0.85
	PTL3		
R1	55.73±7.99	55.78±6.38	42.42±10.97
R2	57.88±7.00	52.86±5.71	47.70±8.13
AV	56.80±1.08	54.32±1.46	45.06±2.69
	PTL2		
R1	47.56±4.29	57.19±2.96	52.83±4.95
R2	57.88±1.56	52.89±4.22	49.52±7.59
AV	52.72±5.16	55.04±2.15	51.17±1.66
AV substrate	57	56	50

4.5. Stability operations of a semi-continuous test

The stability performance of anaerobic digestion operations is analyzed through Figures 13, 14, and 15, highlighting the impact of hydraulic retention time (HRT) on pH levels during digestion of grass waste substrate biomass (GWSB), banana peel waste substrate biomass (BPWSB), and paper waste substrate biomass (PWSB). pH fluctuations are crucial, especially for lignocellulosic substrates like PWSB, with optimal pH ranges of 6.8 to 7.2 [21], [22]. Across different HRTs, pH levels exhibit variations, notably dropping below the optimum range for PWSB under certain conditions. Despite fluctuations, pH levels generally remain within tolerable limits, suggesting suitable conditions for substrate degradation and methane production. Alkalinity ratio indicators (PA, IA, and TA) are employed [18] as stability indicators, with increasing IA:PA ratios indicating potential instability due to volatile fatty acid (VFA) buildup [18]. The IA:PA ratios gradually increase over time, signalling a disturbance in the digesters,

with PA and IA levels reaching 0.42 (BPWSB), 0.41 (GWSB), and 0.45 (PWSB) after four weeks, indicating increasing instability in reactor operations.

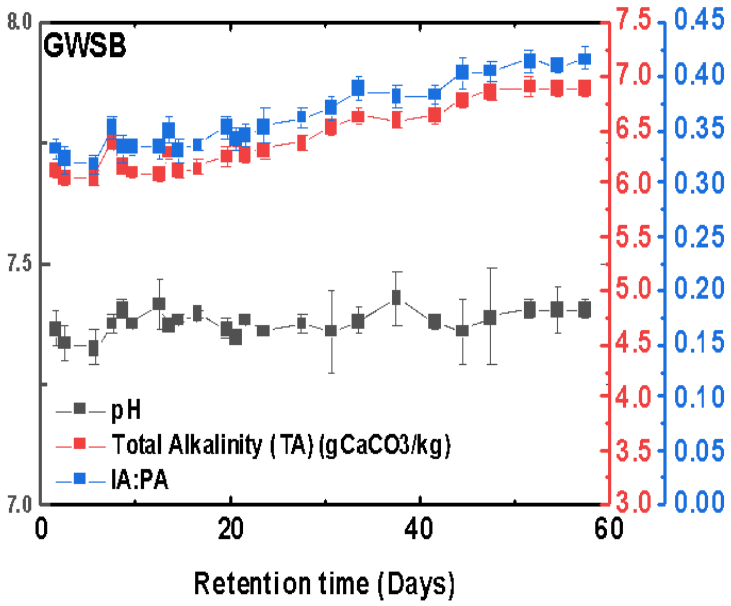


Figure 13. Shows the pH, total and IA:PA alkalinity, for the GWSB digester during the semi-continuous test

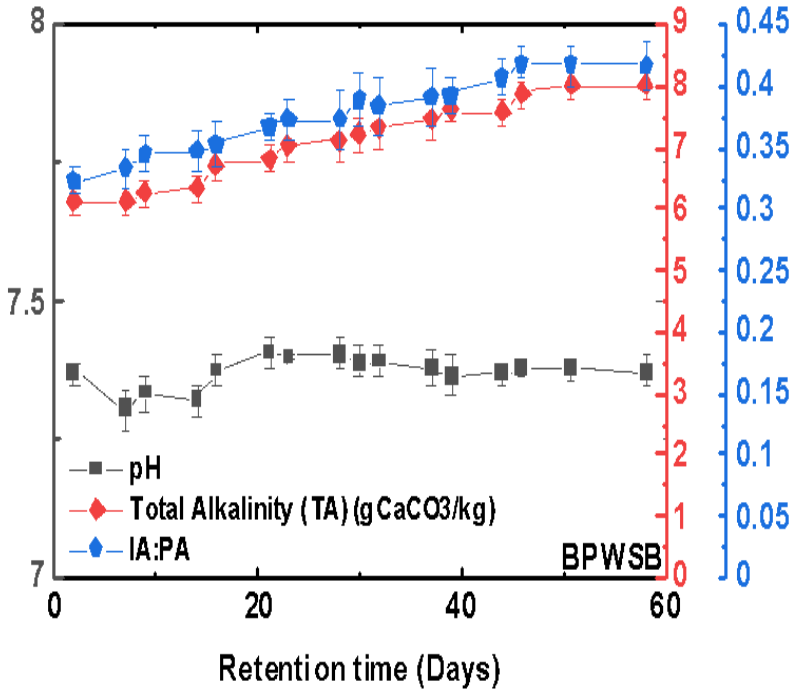


Figure 14. Shows the pH, total and IA:PA alkalinity, for the GWSB digester during the semi-continuous test

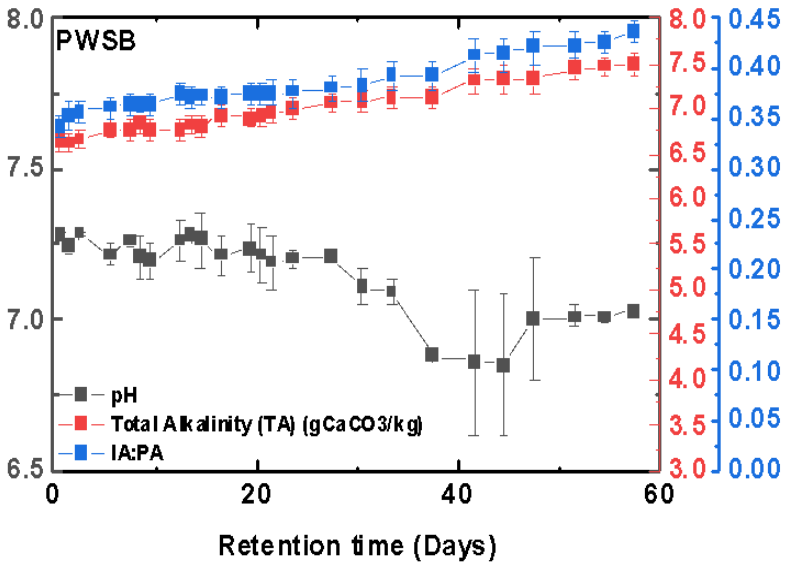


Figure 15. Shows the pH, total and IA:PA alkalinity, for the GWSB digester during the semi-continuous test

4.6. Comparison of experimental and theoretical methane and energy yield

Equations [3.7], [3.8], and [3.9] were utilized to calculate the theoretical gas composition and calorific value based on elemental analysis data of the selected substrate biomass, with the heating value (CV) of the dried substance also determined. Table 14 compares the computed energy values using equations 3.8 and 3.9 of the Dulong formula, showing good agreement despite minor discrepancies. Despite the short hydraulic retention time (HRT) and the rapid degradation rate of the substrate, the semi-continuous tests of the three substrates exhibited enhanced methane output, particularly for BPWSB and PWSB, showing similar methane production patterns. However, differences in methane output between pre-treatment levels (PTLs) were observed, influenced by particle size (PS), with batch tests yielding more methane than semi-continuous tests. This discrepancy may stem from the shorter HRT. The improved methane output in semi-continuous tests may be attributed to increased unit surface area enhancing carbon bioavailability and substrate hydrolysis. Additionally, substrate particle size may affect biological process efficiency [23], [24] - [26], with smaller particles exposing a larger surface area to enzymatic attack, thus enhancing carbon accessibility and hydrolysis [27], [23], [28], [29].

Table 13. 0ne-way analysis of variance of the grass waste substrate biomass

Descriptive Statistics					
	N Analysis	N Missing	Mean	Standard Deviation	SE of Mean
BPWSB PT1	28	0	209.9801	51.99837	9.82677
BPWSB PT2	28	0	228.7962	56.72017	10.7191
BPWSB PT3	28	0	257.6911	61.89203	11.6965
BPWSB PT4	28	0	293.3233	67.63126	12.78111
One Way ANOVA					
	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	3	110913.6	36971.18	10.3231	4.97E-06
Error	108	386791.7	3581.405		
Total	111	497705.3			
Fit Statistics					
	R-Square	Coeff Var	Root MSE	Data Mean	
	0.22285	0.24185	59.84484	247.4477	

Table 14. An overview of digester performance

Substrate	Parameter	Value			
	Pre-treatment levels	PTL1	PTL2	PTL3	PTL4
BPWSB	BMP (Batch test)	245	271	293	332
	Semi continuous test	-	229	253	262
	Theoretical specific methane production	321	327	339	353
	Empirical formula	$C_{32.64}H_{56.57}N_1$			
	Molar mass of empirical formula	463.04			
	% Measured biogas methane CH_4 content	-	55	54	58
	Theoretical %biogas methane CH_4 content	71			
	Calculated CV (energy value) MJ kg-1 VS (equation 3.6)	22			
	Calculated CV (energy value) MJ kg-1 VS (equation 3.7)	20			
	Average batch test specific methane CH_4 production	285			
	Average semi continuous test specific methane CH_4 production	248			
	Average % measured biogas methane CH_4 content of substrate	56			
	Average theoretical specific methane CH_4 production of substrate	353			
	Average digester Ph	7.32			
GWSB	BMP (Batch test)	203	225	255	274
	Semi continuous test	-	242	286	321
	Theoretical specific methane production	268	272	294	301
	Empirical formula	$C_{14.84}H_{26.2}N_1$			
	Molar mass of empirical formula	218.65			
	% Measured biogas methane CH_4 content	-	53	57	61
	Theoretical %biogas methane CH_4 content	70			
	Calculated CV (energy value) MJ kg-1 VS (equation 3.6)	22			
	Calculated CV (energy value) MJ kg-1 VS (equation 3.7)	20			
	Average batch test specific methane CH4.production	239			
	Average semi continuous test specific methane CH4 production	283			
	Average % measured biogas methane CH_4 content of substrate	57			
	Average digester pH	7.33			

Table 14 Continued

Substrate	Parameter	Value			
	Pre-treatment levels	PTL1	PTL2	PTL3	PTL4
PWSB	BMP (Batch test)	231	250	264	274
	Semi continuous test	-	206	240	250
	Theoretical specific methane production (Buswell 1952)	269	273	282	287
	Empirical formula	C_1H_2			
	Molar mass of empirical formula	14.03			
	% Measured biogas methane CH_4 content	-	15	45	55
	Theoretical %biogas methane CH_4 content (Buswell 1952)	72			
	Calculated CV (energy value) MJ kg-1 VS (equation 3.6)	21			
	Calculated CV (energy value) MJ kg-1 VS (equation 3.7)	19			

	Average batch test specific methane CH_4 production	255			
	Average semi continuous test specific methane CH_4 production	232			
	Average % measured biogas methane CH_4 content of substrate	50			
GWSB	BMP (Batch test)	233	260	276	294
	Semi continuous test	-	-	-	-
	Theoretical specific methane production	298	313	321	325
	Empirical formula	$C_{22.62}H_{38.49}N_1$			
	Molar mass of empirical formula	324.5			
	% Measured biogas methane CH_4 content	-	-	-	-
	Theoretical % biogas methane CH_4 content	70			
	Calculated CV (energy value) MJ kg-1 VS (equation 3.6)	21			
	Calculated CV (energy value) MJ kg-1 VS (equation 3.7)	19			
	Average batch test specific methane CH4.production	266			
	Average digester pH	7.33			
- Not measured					

4.7 Comparison of batch and semi-continuous tests

The semi-continuous test spanned 58 days for the three substrate biomass pre-treatment levels, while the batch test lasted 30 days for each pre-treatment level. Contradictory results between the two tests were observed, likely due to differences in organic matter and bacterial activity in the reactor. To compare the batch test with the semi-continuous test, a hydraulic retention time (HRT) of 20 days for GWSB, BPWSB, and 18 days for PWSB was used. Despite these efforts, discrepancies persisted. Table 15 displays the average specific methane output of the three feedstocks across different pre-treatment levels, facilitating comparison between the two tests.

While the batch test indicated higher methane output for BPWSB and PWSB, the semi-continuous test showed higher methane output for all pre-treatment levels of GWSB. These contrasting results stresses the complexity of substrate performance assessment. GWSB generally outperformed BPWSB and PWSB in both tests, suggesting its suitability for anaerobic digestion [48]. The discrepancies observed could be attributed to microbial activity and the particle size paradox, where smaller particles may not necessarily yield higher gas production rates due to factors beyond mean size. The influence of operating conditions, particularly HRT, on biogas methane output was evident [27], [30], [28], [29].

The batch test yielded higher methane output for BPWSB compared to the semi-continuous test, which could be attributed to the greater unit surface area exposed to enzymatic attack in smaller particle sizes. Ultimately, GWSB emerged as the most suitable substrate for anaerobic digestion, while PWSB exhibited lower methane production, potentially due to poor microbial activity owing to its lignin-rich composition [31].

Table 15. Average specific methane output from batch and semi-continuous tests of GWSB, BPWSB and PWSB across the duration of the study

			Substrate Biomass		
	PTLs	HRT (days)	GWSB	PWSB	BPWSB
Batch test	PT4	20	218 ±37 Nml/gVS	251 ±31 Nml/gVS	295 ±34 Nml/gVS
	PT3	20	212 ±36 Nml/gVS	248 ±29 Nml/gVS	259 ±38 Nml/gVS
	PT2	18	117 ±37	228 ±33	223 ±35 Nml/gVS

			Nml/gVS	Nml/gVS	
Semi-continuous test	PT4	20	253 ±29 Nml/gVS	186 ±22 Nml/gVS	235 ±20 Nml/gVS
	PT3	20	228 ±48 Nml/gVS	136 ±40 Nml/gVS	221 ±32 Nml/gVS
	PT2	18	192 ±21 Nml/gVS	164 ±24 Nml/gVS	199 ±22 Nml/gVS

4.8 Comparison of reactors performances for semi-continuous testing

The study's goal is to assess whether there is a variation in methane yield among reactors R1 and R2 from GWSB semi-continuous test results. This hypothesis was tested by comparing the specific methane yields of R1 and R2. An independent samples t-test was used to test this hypothesis. Table 16 reveals that the average specific methane yields in reactor R2 (M=237.2, SD=43.04) are significantly greater than those in reactor R1 (M=214.2, SD=46.05), t (114) =2.78, p=0.006. The magnitude of the effect is medium (Cohen's d =0.516). These results suggest that R2 produced more methane than R1, which could be attributed to a higher microbial population in the digester.

Table 16. Specific methane yield differences in reactors R1 and R2

Group Statistics								
R	N	M	SD	SEM	t	Df	p	Cohen's d
1	58	214.2	46.05	6.05	-2.78	114	0.0064	-0.516
2	58	237.2	43.04	5.65				
R=Reactor=Mean, SD= Std. Deviation, SEM= Std. Error Mean, df= difference								

Using data from the BPWSB semi-continuous test, the study's goal is to determine if specific methane yield differs between reactors R1 and R2. comparing R2 and R1's specific methane yields allowed us to test this hypothesis. This was explored using an independent samples t-test. The average specific methane yields in reactor R1 (M=237.2, SD=43.04) are significantly higher than those in reactor R1 (M=214.2, SD=46.05), as shown in table 17, with a t (114) =6.81, p=<0.006. Cohen's d value of 0.516 indicates a medium-sized effect. Due to a higher microbial population in the digester, these results indicate that R2 produced more methane than R1, which could be explained by this.

Table 17. Specific methane yield differences in reactors R3 and R4

Group Statistics								
R	N	M	SD	SEM	t	df	p	Cohen's d
1	58	241.8	32.27	4.24	6.81	114	<0.001	1.265
2	58	196.7	38.82	5.1				
R=Reactor=Mean, SD= Std. Deviation, SEM= Std. Error Mean, df= difference								

Reactors R1 and R2's Specific methane yields were compared (Table 18). Specific methane yields in reactor R2 (M=164.2, SD=40.61) were, on average, higher than those in reactor R1 (M=159.7, SD=46.12). An independent t-test revealed that this difference was statistically significant; the results were t (114) = 0.558, P = 0.578. The small size of the effect is indicated by the Cohen's d value of 0.261. These results show that R2 produced more methane than R1 due to a higher population of microbe in the digester.

Table 18. Specific methane yield differences in reactors R5 and R6

Group Statistics								
R	N	M	SD	SEM	T	df	p	Cohen's d
1	58	159.7	46.12	6.06	-0.558	114	0.578	0.261
2	58	164.2	40.61	5.33				
R=Reactor=Mean, SD= Std. Deviation, SEM= Std. Error Mean, df= difference								

4.8.1. Performance of the three substrates in terms of methane production

The BPWSB M=225.7 (SD=43.2) specific methane yield was correlated with the GWSB (N=58). Comparatively, the BPWSB M=219.5 (SD=29.5) was associated with the numerically smaller GWSB (N=58). As shown in table 19, an independent samples t test was carried out to determine the hypothesis that the GWSB and BPWSB were associated to statistically significant differences in the mean of the BPWSB. The independent samples t tests revealed statistically significant results, with a value of, t (114) =.942, P=.0348. As a result, the GWSB was associated to a statistically larger mean than the BPWSB. Based on the Cohen's d (1992) guideline, the Cohen's d was estimated at.175, which is a very low value. Table 19. Also displays the mean at 95% confidence intervals. The specific methane yield from the GWSB, M=225.7 (SD=43.2), was correlated with the GWSB (N=58). The numerically smaller PWSB (N=58) was correlated with the PWSB M=161.9 (SD=37.1). The hypothesis that the GWSB and PWSB were associated to statistically significant differences in the mean of the PWSB was examined using an independent samples t test. With a value of, t (114) =8.530, P= <0.001, the independent samples t tests produced statistically significant results. The GWSB was thus the related to a statistically higher mean than the PWSB. The Cohen's d was estimated at 1.584, which large value based on the Cohen's d (1992) guideline. The mean analysed at 95% confidence intervals are shown in Table 19. While the BPWSB (N=58) was correlated with the specific methane yield from the BPWSB, M=219.2 (SD=29.5). The PWSB M=161.9 (SD=37.1) was correlated with the numerically smaller PWSB (N=58). To determine whether the GWSB and BPWSB were connected to statistically significant differences in the mean of the BPWSB, an independent samples t test was conducted. Independent samples t tests produced results that were statistically significant, with a value of t (114) = 9.214, P=. <0.001. Thus, compared to the PWSB, the BPWSB was statistically associated with a higher mean. According to the Cohen's d (1992) formula, the Cohen's d was calculated to be 1.711, which is a large value. Table 19 shows the average.

Table 19. An independent samples t test of the three-substrate used for semi-continuous test

Group Statistics								
S	N	M	SD	SEM	T	df	p	Cohen's d
GWSB	58	225.7	43.2	5.67	0.942	114	0.348	0.175
BPWSB	58	219.2	29.5	3.87				
S	N	M	SD	SEM	T	df	p	Cohen's d
GWSB	58	225.7	43.2	5.67	8.53	114	<0.001	1.584
PWSB	58	161.9	37.1	4.87				
S	N	M	SD	SEM	T	df	p	Cohen's d
BPWSB	58	219.2	29.5	3.87	9.214	114	<0.001	1.711
PWSB	58	161.9	37.1	4.87				
S=Substrate =Mean, SD= Std. Deviation, SEM= Std. Error Mean, df= difference								

4.8.2. Results of the analysis of variance: Average specific methane potential of the GWSB

The study's aim is to determine whether there is significant variance in the average specific methane yield of GWSB based on their chosen PTLs (4-2) from the PSD characterisation. Based on the hypothesis, PTL4 with smaller PSD and more surface area will probably produce more specific methane yield than larger PS with less surface area. The semi-continuous test experimental data were analysed using a between-subjects one-way ANOVA. The results

revealed a significant variation in the specific methane yield between the three (3) pre-treatment levels, with F (2, 55) =13.8 and P=1.42E-05. The effect size is.33% as shown in table 20. Post hoc analysis was performed using Fisher's LSD. The studies reveal that PTL4 (M=253, SD=29.8) has a significantly higher specific methane yield than PTL3 (M=229, SD=49.2) and PTL2 (M=192, SD=21.9). Based on the Fisher's LSD results, PTL3 and PTL2 both differ significantly from PTL4 (M= -24.4, SEM= 11.4, P=B-0.00273) and from PTL2 (M=-60.99, SEM=11.7, P=2.73E-06), respectively, while PT2 differs significantly from PT3 (M= -36.61, SEM= 11.7, P=-0.0036). The results indicate that PTL4 is more likely to produce more specific methane yields than PTL3 and PTL2 because it has more smaller PS and more surface area. This could suggest that PTL4 of the GWSB produces a higher specific methane yield than other methods owing to the activity of the microbial population in the reactor. As shown in figures 16 and 17, the results were presented using a box chart and post hoc test fisher LSD.

Table 20. Results of the analysis of variance: average specific methane potential of the GWSB

ANOVA One Way								
Descriptive Statistics of GWSB								
	N Analysis	N Missing	Mean	Standard Deviation	SE of Mean			
PT4	20	0	253.03	29.8	6.7			
PT3	20	0	228.66	49.2	10.9			
PT2	18	2	192.04	21.9	5.2			
Overall ANOVA								
	DF	Sum of Squares	Mean Square	F Value	Prob> F	η^2		
Model	2	35507.9	17753.9	13.8	1.42E-05	.334		
Error	55	70906.7	1289.2					
Total	57	106414.6						
Fit Statistics								
	R-Square	Coeff Var	Root MSE	Data Mean				
	0.334	0.16	35.9	225.7				
				Prob	Alpha	Sig	LCL	UCL
Homogeneity of Variance Test Levene's Test (Absolute Deviations)								
	DF	Sum of Squares	Mean Square	F Value	Prob> F			
	Model 2	2127.9	1063.9	1.67	.1972			
	Error 55	34985.9	636.1					
Means Comparison								
	MeanDiff	SEM	t Value	Prob	Alpha	Sig	LCL	UCL
PT3 PT4	-24.38	11.4	-2.15	0.036	0.05	1	-47.13	-1.62
PT2 PT4	-60.99	11.7	-5.23	2.73E-06	0.05	1	-84.37	-37.61
PT2 PT3	-36.61	11.7	-3.14	0.00273	0.05	1	-59.99	-13.24

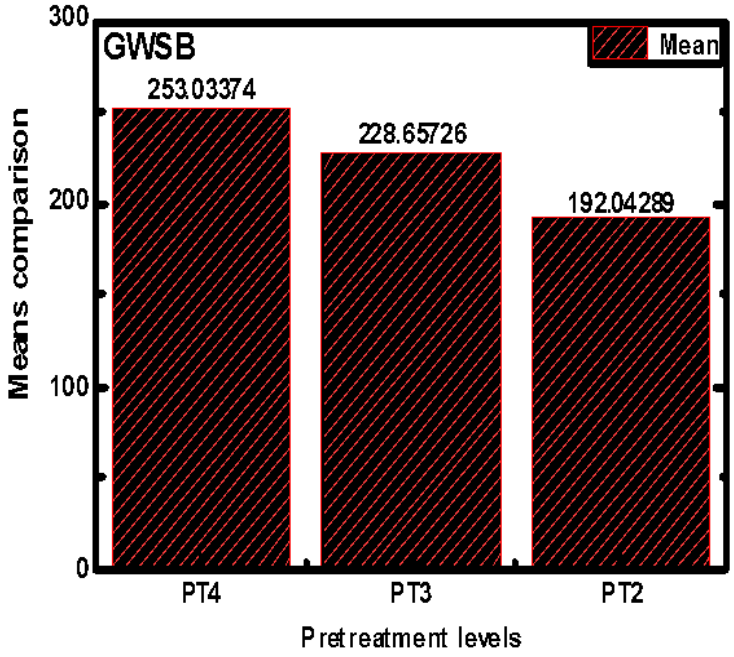


Figure 16. Means bar chart of the GWSB average specific methane yield's variation

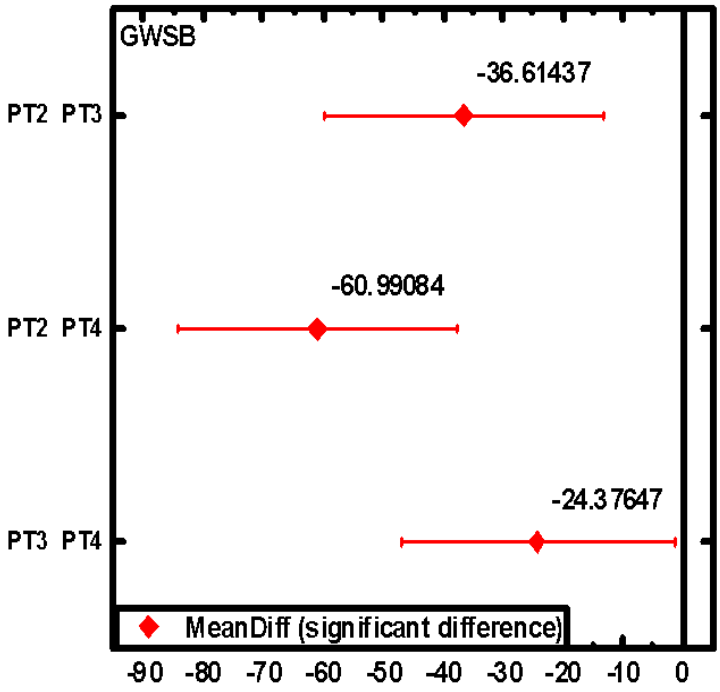


Figure 17. Means Comparison plot using fisher LSD

4.8.3. Results of the analysis of variance: average specific methane potential of the BPWSB

The purpose of the study is to determine how well the chosen PTLs (4-2) from the PSD characterisation represent a significant variation in the average specific methane yield of BPWSB. According to the hypothesis, PTL4 will likely produce a higher specific methane yield than larger PS with lower surface area. Using a between-subjects one-way ANOVA, the experimental data from the semi-continuous test were analysed. With F (2, 55) = 9.10 and P=3.87E-04, the results reveal a significant difference in the specific methane yield between the three (3) pre-treatment levels. The effect is 25% in size. Fisher's LSD was used for post hoc analysis as shown in table 21. The study reveals that PTL4 (M=235.5, SD=20.9) has a significantly higher specific methane yield when compared to PTL3 (M=220.8, SD=32.8) and PTL2 (M=199.5.8, SD=22.2). Fisher's LSD results showed that PTL2 differ significantly from PTL4 (M= -35.9, SEM=8.45), t=-4.25, P= 8.26E-05) and PTL2 differ significantly from PTL3 (M= -21.2, SEM=8.45), t=- 2.5, P= 0.01494), respectively, while PT2 differs significantly from PT3 (M= -36.61, SEM= 11.7, P=-0.0036). PTL3 also have no significant variation from PTL4 (M= -14.7, SEM=8.22), t=-1.79, P= 7.94E-02). As a result of its larger surface area and smaller PS than PTL3 and PTL2, PTL4 is more likely to produce more specific methane yields, according to the results. This might imply that PTL4 of the BPWSB produces a higher specific methane yield than those of other processes because the microbe in the reactor is active. Figures 18 and 19 present the findings using a SD as error and a post hoc fisher LSD plot, respectively.

Table 21. Results of the analysis of variance: average specific methane potential of the BPWSB

ANOVA One Way								
Descriptive Statistics of BPWSB								
	N Analysis	N Missing	Mean	Standard Deviation	SE of Mean			
PT4	20	0	235.4598	20.94647	4.68377			
PT3	20	0	220.7538	32.82749	7.34045			
PT2	18	2	199.515	22.24403	5.24297			
Overall ANOVA								
	DF	Sum of Squares	Mean Square	F Value	Prob> F	η^2		
Model	2	12310.84	6155.419	9.0951	3.87E-04	2		
Error	55	37223.12	676.784			55		
Total	57	49533.96				57		
Fit Statistics								
	R-Square	Coeff Var	Root MSE	Data Mean				
	0.24853	0.11866	26.01507	219.2335				
Means Comparison								
	MeanDiff	SEM	t Value	Prob	Alpha	Sig	LCL	UCL
PT3 PT4	-14.7061	8.22669	-1.7876	7.94E-02	0.05	0	-31.1927	1.7806
PT2 PT4	-35.9448	8.45212	-4.25276	8.26E-05	0.05	1	-52.8832	-19.0064
PT2 PT3	-21.2388	8.45212	-2.51283	0.01494	0.05	1	-38.1772	-4.30034
Homogeneity of Variance Test Levene's Test (Absolute Deviations)								
	DF	Sum of Squares	Mean Square	F Value	Prob> F			
Model	2	179.4294	89.71471	0.29927	0.74256			
Error	55	16487.72	299.7767					

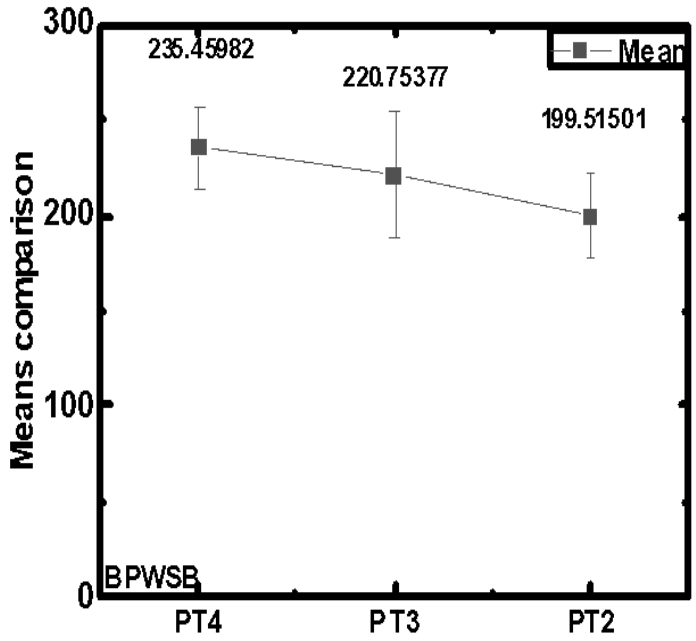


Figure 18. Means plot SD as error for the BPWSB average specific methane yield's variation

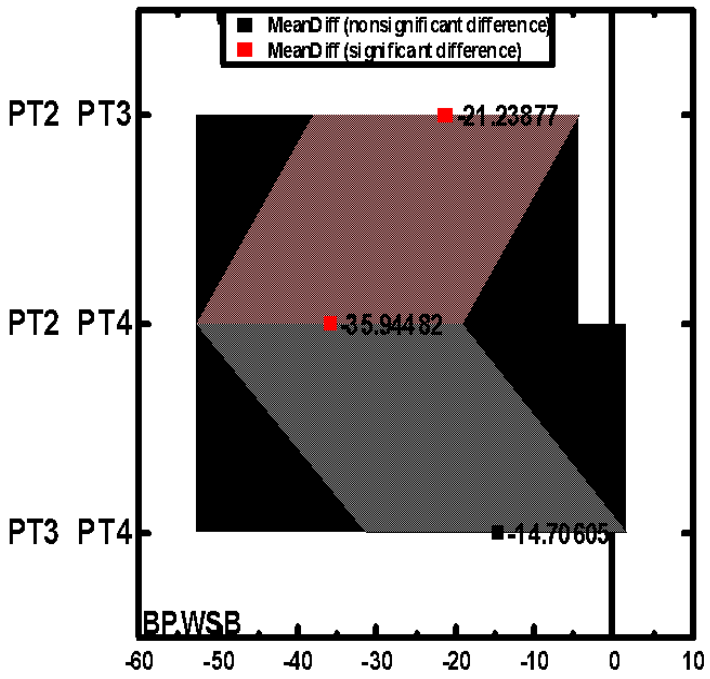


Figure 19. Means comparison plot using fisher LSD

4.8.4 Results of the analysis of variance: average specific methane potential of the PWSB

The goal of the study is to evaluate how well the PSD characterisation's PTLs (4-2) chosen PTLs (4-2) represent a significant variation in the average specific methane yield of PWSB. The hypothesis suggests that PTL4 will probably yield a greater specific methane yield than larger PS with a smaller surface area. The experimental data from the semi-continuous test were analysed with a between-subjects one-way ANOVA. As shown in table 22, the results revealed a significant difference in the specific methane yield between the three (3) pre-treatment levels, with F (2, 55) =12.9, P=2.62E-05. Post hoc analysis was performed using Fisher's LSD. According to the data analysis, PTL4 (M=185.9, SD=23.0) has a significantly higher average specific methane yield than PTL2 (M= 164.1, SD= 25.3) and PTL3 (M= 136.0, SD= 41.3). There is .32% influence. Fisher's LSD analysis indicate that PTL3 and PTL2 significantly differ from PTL4 (M= -24.4, SEM= 11.4, P=B-0.00273) and from PTL2 (M= -60.99, SEM=11.7, P=2.73E-06), respectively, while PT2 significantly differs from PT3 (M= -36.61, SEM= 11.7, P=-0.0036). The results suggest that PTL4 is more likely to yield more specific methane yields due to its greater surface area and smaller PS than PTL3 and PTL2. Because the microbe in the reactor is active, this could mean that PTL4 of the PWSB has a greater specific methane yield than those of other processes. PTL3 and PTL2, on the other hand, which are comparable in that they both contain larger PS and less surface area, are more likely to yield the same amount of a specific methane. A box chart and a means comparisons plot fisher were used to display the results, as shown in figures 20 and 21.

Table 22. Results of the analysis of variance: average specific methane potential of the PWSB

ANOVA One Way								
Descriptive Statistics of PWSB								
	N Analysis	N Missing	Mean	Standard Deviation	SE of Mean			
PT4	20	0	185.8772	23.03612	5.15103			
PT3	20	0	136.0314	41.32925	9.2415			
PT2	18	2	164.0909	25.26574	5.95519			
Overall ANOVA								
	DF	Sum of Squares	Mean Square	F Value	Prob> F			
Model	2	24968.2	12484.1	12.86087	2.62E-05			
Error	55	53388.71	970.7039					
Total	57	78356.91						
Fit Statistics								
	R-Square	Coeff Var	Root MSE	Data Mean				
	0.31865	0.19241	31.15612	161.9277				
Means Comparison								
	MeanDiff	SEM	t Value	Prob	Alpha	Sig	LCL	UCL
PT3 PT4	-49.8458	9.85243	-5.05924	5.01E-06	0.05	1	-69.5905	-30.1011
PT2 PT4	-21.7864	10.12241	-2.15229	0.03578	0.05	1	-42.0721	-1.50059
PT2 PT3	28.05948	10.12241	2.77202	0.00759	0.05	1	7.77372	48.34524
Homogeneity of Variance Test Levene's Test (Absolute Deviations)								
	DF	Sum of Squares	Mean Square	F Value	Prob> F			
Model	2	1832.04	916.0198	2.51949	0.08975			
Error	55	19996.53	363.5732					

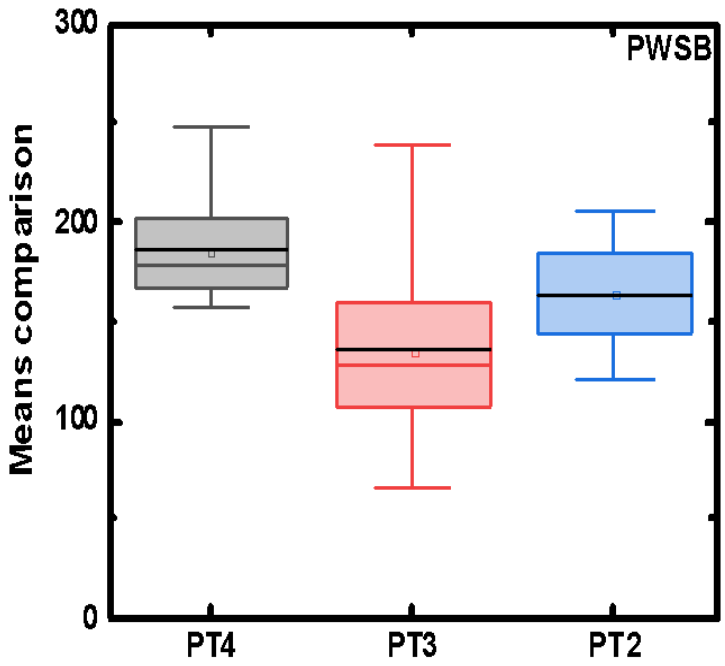


Figure 20. Means boxplot of the PWSB average specific methane yield's variation

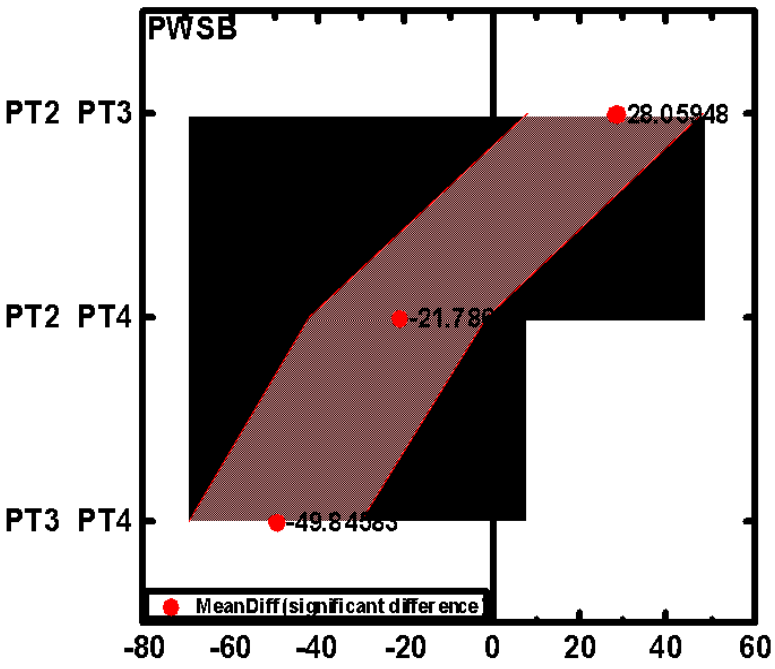


Figure 21. Means Comparison plot using fisher LSD.

4.9. A comparison of the specific methane yields of four substrate pre-treatment levels used in the batch test

The main reason for the comparison was to ascertain whether there are any notable differences between the four PTLs for substrates that were selected to be used in a batch test. It was proposed that BPWSB are more likely to produce greater methane in their various PTLs than others substrate biomass. The data was collected over a 25-day test period for all substrates for the analysis. This is because some substrate's data was recorded for longer than 25 days. After that, a between-subjects one-way ANOVA was used to analyse the data (Table 23 and figure 22). The Fisher LSD was used in the post-hoc analysis. The results revealed a significant variation between the four PTL1-4 of each substrate, with F (15,400) =7.22 and 4.01E-14. Specific methane yields differ significantly between the four pre-treatment levels, according to the studies, as shown in appendix C1. TWSB PTL1 had a higher specific methane yield than the other substrates (M=210.07, SD=55.8), while GWSB PTL1 had the lowest (M=210.07, SD=55.8). According to the study results, the specific methane yields for BPWSB, TWSB, and PWSB are comparable but not identical. The highest specific methane yield was produced by BPWSB for PTL2 (M=225.6, SD=57.7), while the lowest was produced by GWSB (M=181.2, SD=51.9). The results suggest that the specific methane yields for BPWSB, TWSB, and PWSB are similar but not identical. Again, BPWSB PTL3 (M=255, SD=63.5) differed significantly from the others, whereas GWSB had the lowest specific methane yield (M=211.7, SD=56.6). Likewise, BPWSB PTL4 outperformed the other substrates (M=290.3, SD=69.3), while GWSB PTL4 has the least (M=218.3, SD=69.4).

Table 23. Results of the analysis of variance between the four substrates used in the batch test for a period of 25 days

ANOVA One Way					
Descriptive Statistics					
	N Analysis	N Missing	Mean	Standard Deviation	SE of Mean
	N Analysis	N Missing	Mean	Standard Deviation	SE of Mean
GWSBPTL1	26	0	158.3043	51.48986	10.09799
BWSBPTL1	26	0	207.244	53.02007	10.39809
PWSBPTL1	26	0	204.5089	53.53726	10.49952
TWSBPTL1	26	0	210.0675	55.84928	10.95294
GWSBPTL2	26	0	181.2355	51.89785	10.17801
BWSBPTL2	26	0	225.5784	57.65218	11.30652
PWSBPTL2	26	0	225.2383	54.58297	10.7046
TWSBPTL2	26	0	221.9227	63.1478	12.3843
GWSBPTL3	26	0	211.6531	56.57955	11.09616
BWSBPTL3	26	0	254.9541	63.46653	12.44681
PWSBPTL3	26	0	241.9266	56.20291	11.0223
TWSBPTL3	26	0	243.6956	63.3382	12.42164
GWSBPTL4	26	0	218.0484	58.4785	11.46858
BWSBPTL4	26	0	290.333	69.35201	13.60105
PWSBPTL4	26	0	245.2892	57.69149	11.31423
TWSBPTL4	26	0	251.3372	71.54467	14.03106
	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	15	375970.6	25064.7	7.22483	4.01E-14
Error	400	1387699	3469.247		
Total	415	1763669			
	R-Square	Coeff Var	Root MSE	Data Mean	
	0.21318	0.26241	58.90031	224.4585	

	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	15	9939.717	662.6478	0.33693	0.99121
Error	400	786679.8	1966.699		

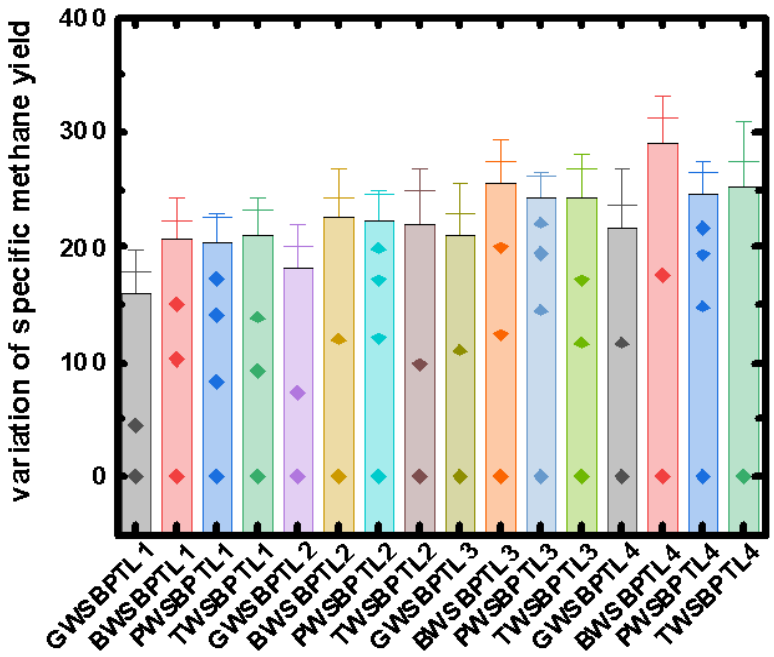


Figure 22. Means bar plot of the average specific methane yields of four substrate from batch test

5. Conclusions

The expected outcomes of semi-continuous test were as follows:

- It is expected that after applying different feedstock enhancement solutions to the various feedstocks investigated, such as the pre-treatment, it should show a viable method for upgrading the biogas yield of the feedstock and thereby enhancing the overall anaerobic digestion (AD) process.
- It is expected that increasing the pre-treatment intensity will result in a greater specific yield of biogas since decreasing the particle size will increase the total surface area of the solids by the opening of the compact structure leading to higher biodegradability and an increase in the biogas [49], [50], [51].
- It is expected that the effect of pre-treatment on the maximum OLR and maximum volumetric biogas production could be to increase or decrease the maximum biogas production from a continuous system, depending on which is the predominant effect, or eventual failure mechanism. More intensive pre-treatment could lead to an increased tendency for foaming to occur in the system, thus reducing the maximum biogas production. However, the increased biodegradability of the material subject to pre-treatment could lead to enhancement of the maximum biogas production.
- The increase in pre-treatment intensity (i.e., PS1-PS3) will result in a reduced tendency to foaming. This is because several scientific researchers [20], [33], [14], [34] have reported that organic overloading of digesters can be a reason for foaming. This is because of the excess compounds not being degraded by the bacteria within the digesters, thereby leading to the potential accumulation of hydrophobic or surface-active by-products that will promote foaming. Hence, increasing the substrate surface area through pre-treatment

intensity will assist in providing more access to microbial degradation, since the rate and degree of degradation increases after size reduction.

The reactions to changing feed when PTLs with less surface area PS are fed, along with the effects of short HRT on the methane content and specific methane yield, are the most obvious similarities between the reactors R1 to R6. The methane production profiles of the PWSB differed significantly. While PS with more surface area (PTL4) outperformed the other three PTLs with lesser surface area, GWSB outperformed both BPWSB and GWSB. Also, for all substrates, the spike in methane yield was greater for the GWSB compared to the other substrates, as well as between PTL4 and the other two PTLs.

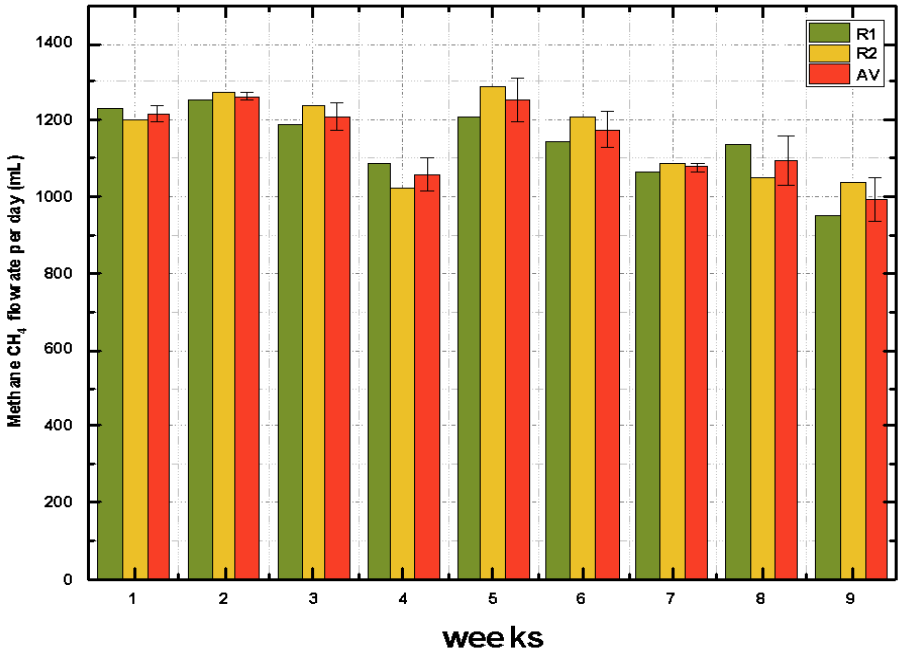
The changeover from large surface area PTL4 to smaller surface area PTL3 and PTL2 feed affected the process stability indicators such as pH, total and partial alkalinity, and IA/PA ratio, as well as shorter HRT, which reduce the substrate destruction and biogas in methane output.

One-way ANOVA and Post-hoc Fisher LSD were used to analyse the experimental data from the semi-continuous test, and they showed that PTL4 produced more specific methane yield than PTL3 and PTL2 because of its greater surface area and smaller PS. On the other hand, PTL3 and PTL2, which are comparable in that they both contain larger PS and have less surface area, are more likely to produce the same amount of a specific methane yield for all substates.

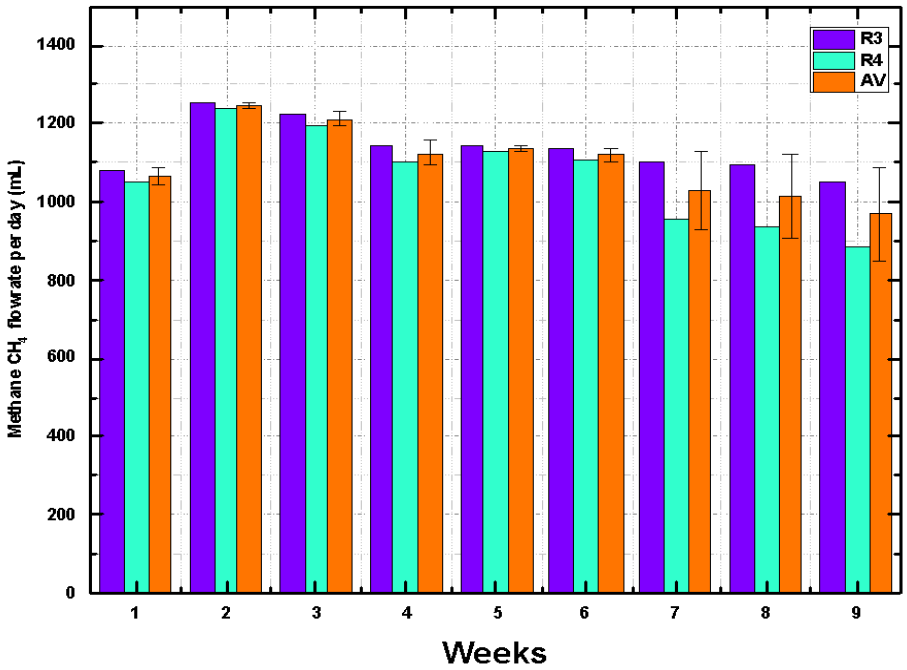
The increasing pre-treatment intensity of substrates led to a greater specific surface area while enhancing the process' output and increasing the production of biogas by decreasing the size of the particle size and increasing the total surface area of the substrates. The highest amount of biogas produced increased because of the pre-treated material's greater biodegradability, which was influenced by the hydraulic retention time (HRT) and change in feed. The results reveal that intensive pre-treatment had no detrimental effects on the anaerobic digestion system (e.g., foaming). The study showed that an increase in pre-treatment intensity causes a decrease in the tendency to foam (PS2-PS4).

Appendices

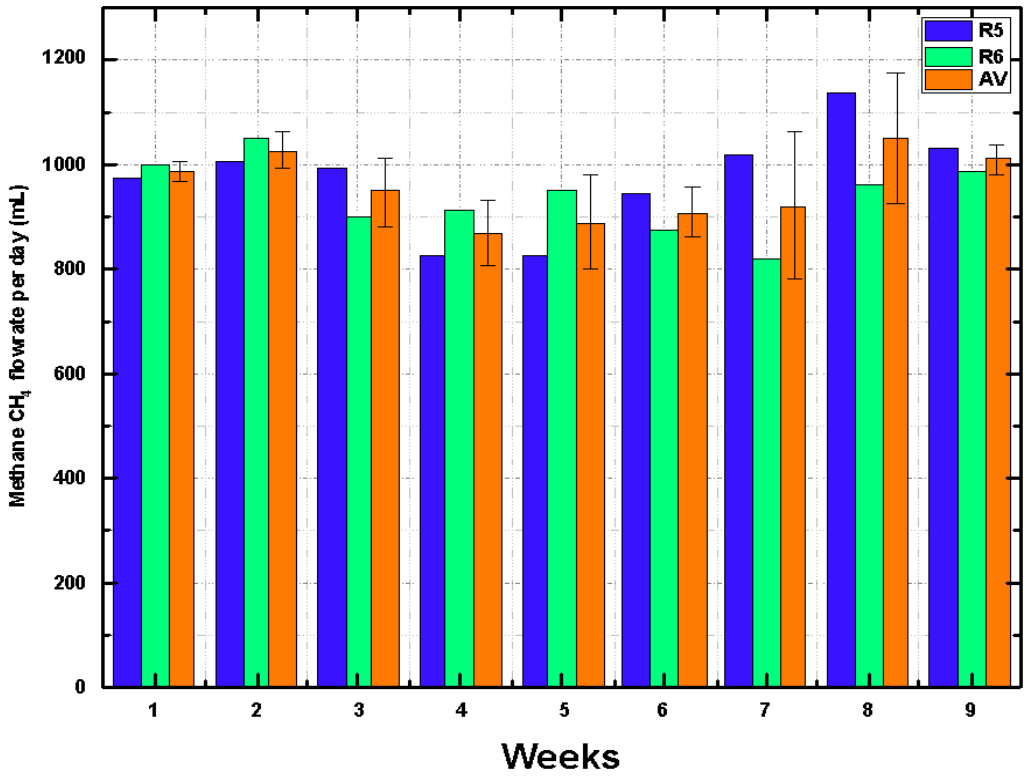
Semi Continuous Testing



Appendix A1 Daily average banana peel waste substrate biomass (GWSB) methane flow rate above 1149ml/day for 9 weeks,



Appendix A2 Daily average paper waste substrate biomass (B**PWSB**) methane flow rate above 1149ml/day for 9 weeks



Appendix A3 Daily average paper waste substrate biomass (**PWSB**) methane flow rate above 955ml/ day for 9 weeks.

B1 = Calculation of calorific value modified Dulong formulas

$337C+1419(H-1419\frac{0}{8}+93S+23.26N$

GWSB= 337(39.19) =13207
1419 (5.8) - $\frac{0}{8}$ =8230
23.26(3.08) =72
Energy value =337(39.19) +1419(5.8-1419$\frac{0}{8}$ +23.26(3.08) = 22 MJ/kgVS
BPWSB= 337(40.01) =13483
1419 (5.82) - $\frac{0}{8}$ =8259
23.26(1.43) = 33.3
Energy value =337(40.01) +1419(5.82-1419$\frac{0}{8}$ +23.26(1.43) = 22 MJ/kgVS
PWSB= 337(38.19) =12870
1419 (5.57) - $\frac{0}{8}$ =7904
23.26(0) = 23.26
Energy value =337(38.19) +1419(5.57-1419$\frac{0}{8}$ +23.26(0) = 21MJ/kgVS
TWSB= 337(39.55) =13328
1419 (5.65) - $\frac{0}{8}$ =8017
23.26(2.04) = 48
Energy value =337(38.19) +1419(5.65-1419$\frac{0}{8}$ +23.26(2.04) = 21 MJ/kgVS
0R.

B2 =34.1C+102H+6.3N+19.1(0)-9.850)/100

GWSB =34.1(39.19) +102(5.8) +6.3(3.08) +19.1S-9.85(0)/100 =20 MJ/kgVS
BPWSB =34.1(40.01) +102(5.82) +6.3(1.43) +19.1(0) -9.85(0)/100 =20 MJ/kgVS
PWSB =34.1(38.19) +102(5.57) +6.3(0) +19.1(0)-9.85(0)/100 =19 MJ/kgVS
TWSB =34.1(39.55) +102(5.65) +6.3(2.04) +19.1(0)-9.85(0)/100 =19 MJ/kgVS

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