Effect of Particle Size Distribution on Kinetics and Overall Degradation in Anaerobic Digestion of Waste Biomass

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Abstract: Particle size distribution in pre-treated organic substrates significantly impacts microbial fermentation efficiency, with smaller particles offering greater specific surface area, potentially reducing retention times and operational costs, particularly beneficial for remote and urban areas. Laboratory tests compared manual chopping, shredding, grinding, and mincing techniques, finding manual methods more effective than shredders at pre-treatment level 1. Non-treated tomato waste showed the highest fine particle distribution (41%), contrasting with grass waste (< 2.9 mm), banana peel waste (< 2 mm), and paper waste (< 3 mm). Combining mincing, grinding, and extended processing increased methane output, particularly evident in higher pre-treatment levels with enhanced surface area. Among substrates, banana peel waste biomass (BPWSB) yielded the most methane (332 ± 36 Nml/gVS, 67% VSR), while grass waste biomass (GWSB) in semi-continuous tests produced 253 ± 29 Nml/gVS. The Biochemical Methane Potential (BMP) kinetic models consistently favored first-order kinetics over the modified Gompertz model across all substrates, attributing higher k-values to PTL4 due to its large surface area: BPWSB (0.59), GWSB (0.339), PWSB (0.59), and TWSB (0.59). These findings emphasized slower decay of larger particles and more rapid degradation of smaller fractions, crucial for optimizing biogas production efficiencies.

Keywords: Anaerobic, methane, particle size, biomass, banana peel grass, paper, tomato substrate, biochemical methane potential.

1. Introduction

Energy plays an important role in economic development, and achieving sustainable development hinges on a well-developed energy sector. However, attaining a balanced energy mix to address global warming is particularly challenging in developing nations like Nigeria. Many people in rural areas rely on firewood for basic energy needs, causing air pollution and health problems. Meanwhile, dependence on fossil fuels like coal, oil, and natural gas leads to environmental degradation, intensifying global warming and climate change, threatening both human and animal well-being.

Nigeria, Africa's largest economy and most populous nation, faces significant hurdles in providing opportunities for its citizens due to limited access to education and healthcare, resulting in diminished productivity expectations. Weak job creation and entrepreneurial prospects contribute to a poverty rate of 38.9%, with 87 million Nigerians living below the poverty line. Spatial inequality, regional disparities, low state capacity, and widespread insecurity persist, while infrastructure gaps hinder economic integration and worsen challenges [1]. These issues not only pose environmental risks but also contribute to economic and social problems that require urgent attention from the nation.

In Nigeria, over half the population lacks access to clean energy, and even those connected to the national grid experience frequent power interruptions due to financial and technical challenges in the energy sector [2].

Consequently, many individuals and businesses rely on costly self-generated energy from diesel generators [3], [4], leading to environmental hazards and highlighting the need for cleaner energy alternatives. Bridging the historic gap in power demand necessitates the adoption of renewable energy technologies. Energy not only acts as a shield against poverty resulting from economic decline but also drives production in sectors such as manufacturing, agriculture, commerce, and mining [5]. With approximately 61% of the population living below the poverty line, energy plays a crucial role in supporting education, transportation, and communication. Despite significant economic difficulties and a lack of access to clean energy, there is potential for improvement, particularly in rural communities, across many developing nations [6].

Biogas technology holds immense potential for helping developing nations improve access to clean energy by producing methane, a valuable energy source, and generating beneficial soil conditioners and fertilizers through the anaerobic digestion (AD) process [7]. Its small-scale operation and low capital requirements make it particularly advantageous for decentralization and deployment in remote, rural, and urban areas. This technology presents a clear opportunity to address energy challenges in Nigeria, emphasizing the need to enhance understanding of AD process operation and optimization. This study focuses on investigating the impact of particle size distribution on system kinetics and overall degradation during AD of waste biomass, examining various mechanical equipment and four selected feedstocks (banana, grass, paper, and tomatoes).

The AD process involves the decomposition of organic matter within a digester, yielding biogas along with solid and liquid residues [8]. Biogas mainly consists of methane (CH_4) and carbon dioxide (CO_2), with minor quantities of other components such as hydrogen (H_2), hydrogen sulphide (H_2S), ammonia (NH_3), nitrogen, carbon monoxide, oxygen, and siloxanes [9]. Unlike aerobic systems, which require oxygen for reaction, AD occurs in the absence of oxygen. The general equation for anaerobic digestion is Organic matter + Combined Oxygen + Anaerobic microbes → CH_4 + CO_2 + Other end-products. AD involves four dynamic biological stages: hydrolysis, acidogenesis, acetogenesis, and methanogenesis, each characterized by distinct methodologies [10] – [12].

Interspecies hydrogen transfer refers to the symbiotic relationship between hydrogen-utilizing archaea, such as methanogens, and organisms that produce hydrogen through fermentation. Methanogenic archaea rely on fermenters/acetogens to supply them with hydrogen, carbonate, and acetic acid as substrates, while acetogens depend on methanogens to consume hydrogen because their metabolic processes are only viable under extremely low hydrogen levels. This mutualistic interaction enables efficient syntrophic cooperation between different microbial groups [13], [14].

Anaerobic digestion systems offer several advantages over aerobic systems, primarily due to their low energy input requirements and the production of valuable biogas. Angelidaki and Sanders [15] emphasize the importance of optimizing the interaction between enzymes and substrate for efficient hydrolysis. Various pre-treatment techniques, including physical, chemical, biological, and combinations thereof, have been explored to enhance microbial degradation of complex waste. Among these methods, reducing substrate particle size through pre-treatment has shown promise by increasing the surface area available for microbial activity, thereby enhancing biogas production [16] - [19]. Pre-treatment of lignocellulosic materials induces significant structural changes, breaking molecular bonds and increasing surface area [20], [21]. Therefore, it is imperative to study different particle sizes and shapes fed into anaerobic digester systems to understand how they impact AD process performance.

2. Literature Review

2.1. Phases of the anaerobic digestion process

Hydrolysis is a crucial process in anaerobic digestion, wherein complex organic matter is broken down into simpler soluble molecules. Enzymes, produced by anaerobic bacteria as they feed on the substrate, catalyse this conversion, facilitating the breakdown of complex compounds into more accessible forms [22]. The substrate for anaerobic digestion comprises carbohydrates, proteins, lipids, or composite compounds like sludge or yeast. Upon degradation, it yields long-chain fatty acids (LCFA), amino acids, and monosaccharides as primary products. Esposito *et al.* [23] outlined three primary pathways of enzymatic hydrolysis: Anaerobic bacteria release catalysts into the surrounding fluid, where they either adsorb onto a molecule or react with a soluble substrate. Anaerobic bacteria attach to particles and produce enzymes near the surface. Upon enzymatic reaction, the microorganisms utilize the

soluble products released. Anaerobic organisms possess attached catalysts, which may act as a vehicle receptor to transport substrates into the cell interior. This method necessitates bacteria to adsorb onto the molecule's surface [24] – [26]. The hydrolysis of complex particulate substrates involves a two-phase reaction, according to Vavilin *et al.* [25]. The initial phase entails bacterial colonization, during which hydrolytic bacteria cover the substrate's surface, with the reaction rate dependent on the contact area available. The reaction rate is influenced by factors such as substrate composition, solid concentration, and digester hydraulics, all of which impact the efficiency of hydrolysis [27], [28], [29].

The enzymatic hydrolysis process involves several steps: Catalyst production may decrease with excessive soluble substrate availability, impacting the process [30]. Microbes produce enzymes, which are then transferred to the bulk. Enzymes diffuse from the bulk to the feedstock particle. Adsorption processes are constrained to the substrate particle's surface area. The reaction rate is influenced by enzyme concentration and substrate surface area. Products diffuse from the particle to the bulk. Catalyst deactivation can occur excessively if there's a shift from optimal pH and temperature [24].

Several complex hydrolysis kinetic models including various steps have been proposed, as depicted in (Figure 1) [29]. However, validating these intricate models has proven challenging. The simplest and most commonly employed model to describe the hydrolysis process is the first-order kinetic rate model, initially proposed by Eastman and Ferguson [31].

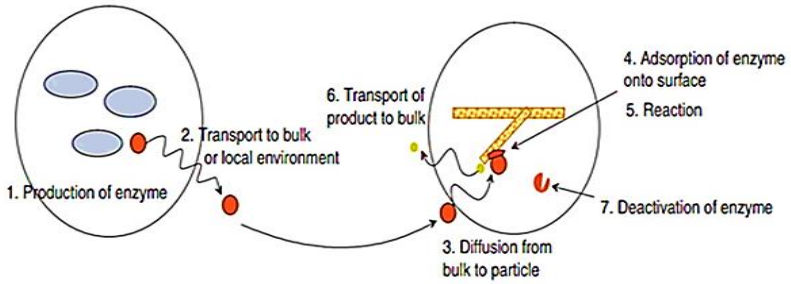


Figure 1. Main steps in enzymatic hydrolysis [29]

The first-order kinetic model, proposed by Eastman and Ferguson, offers an empirical representation of the collective microbial activities within the AD process. As part of hydrolysis, particulate substrates encounter hydrolytic microbial cells, triggering the action of enzymes like hydrolases and lapses. These enzymes facilitate the breakdown of polymeric bonds, resulting in the formation of shorter-chain molecules [14], [24], [29], [32]. The reactions involved in the hydrolysis of lipids and proteins are depicted in figures 2 and 3, respectively.

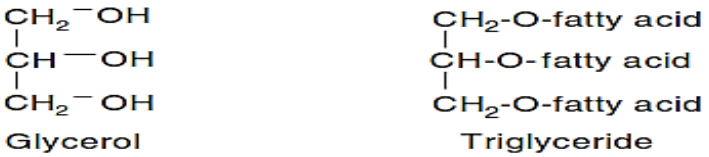


Figure 2. Glycerol and triglycerides [29]

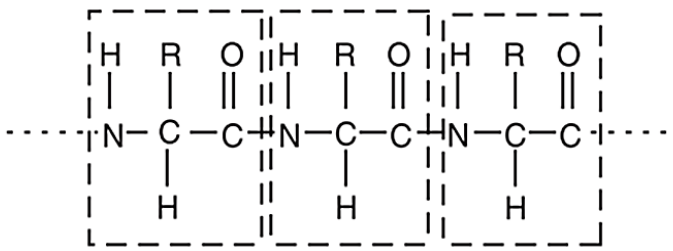


Figure 3. Protein chain and amino acids linked by amide group [29]

Hydrolysis, particularly in the degradation of complex organic materials like lignocellulosic biomass found in food waste and manure, is influenced by substrate biodegradability [33], [34]. The rate of cellulose degradation is influenced by enzymatic activity during hydrolysis and depends on factors such as cellulose polymer properties, environmental conditions, and high-temperature pre-treatment [35].

In acidogenesis, the products of hydrolysis undergo further breakdown into simpler and more easily degradable fractions. This stage, the second phase of the anaerobic digestion (AD) process, is also the fastest [14], [33], [36]. Acidogenesis is characterized by the production of volatile fatty acids (VFA), alongside ammonia, CO_2, H_2, and other by-products, facilitated by acidogenic bacteria. Among the organic acids produced, acetate holds particular importance as it serves as a direct substrate for methanogenic organisms.

Acetogenesis, the third stage of the AD process, involves acetogens consuming VFAs and LCFAs generated from lipid hydrolysis, producing acetic acid, hydrogen, and CO_2. Acetogens cooperate with methanogenic bacteria in interspecies hydrogen transfer, while homoacetogenic bacteria convert CO_2 and H_2 into acetic acid. Acetogenic bacteria are sensitive to environmental factors like temperature and pH levels.

Methanogenesis, the final phase of anaerobic digestion, involves methanogenic archaea synthesizing methane. This synthesis occurs by breaking down acid molecules to produce carbon dioxide and methane. Also, methane synthesis can also happen through the reduction of carbon dioxide (CO_2) with hydrogen [37]. Anaerobic bacteria and acetogens supply methanogens with essential substrates such as hydrogen, carbonate, and acetic acid. In turn, methanogens support acetogens by maintaining a low concentration of dissolved hydrogen, which renders the degradation of organic matter thermodynamically favourable [14], [38], [39].

2.2. The end-products of the anaerobic complex particulate matter

Biogas, generated through the anaerobic digestion (AD) process, results from the breakdown of organic materials by four types of anaerobic bacteria: hydrolytic, acidogenic, acetogenic, and methanogenic. Its primary constituents, methane (CH_4) and carbon dioxide (CO_2), determine its energy content. Biogas may also contain minor components such as ammonia (NH_3), hydrogen (H_2), nitrogen, hydrogen sulphide (H_2S), oxygen, and volatile siloxanes. To use biogas as fuel, it needs scrubbing or upgrading to biomethane, especially to remove toxic elements like sulphide (H_2S) and inhibitive elements like siloxanes, originating from AD of wastewater and household waste [40].

Digestate refers to the residual material left after organic feedstock undergoes anaerobic digestion (AD) to produce biogas. This material can be utilized directly or subjected to various refinement processes. Typically, digestate comprises both solid and liquid components, which are separated during AD into materials with different dry matter contents. For example, fibre sludge is one such solid component. The inhibitory properties of digestate depend on the nature and source of the substrate used in the AD process. High levels of ammonia in digestate can undergo conversion to nitrates, enriching the nutrient content and making the digestate suitable for use as fertilizer [40].

2.3. Factors affecting the digestion of the anaerobic substrate biomass

The digestion of anaerobic substrate biomass is influenced by various factors that impact the stability of anaerobic digestion (AD) kinetics. These factors operate at both the input and output of the reactor. Key operational measures, pre-treatment methods of feedstocks, and post-treatment of end-products are crucial in anaerobic food chain production. Factors affecting reactor inputs comprise pH, temperature, organic loading rate (OLR), mixing, retention time, and physical, chemical, and biological treatments. Meanwhile, factors influencing reactor outputs include by-products such as biogas and digestate [41], [42] – [44].

Anaerobic digestion operates within various temperature ranges, including psychrophilic (10 to 25°C), mesophilic (30 to 40°C), and thermophilic (50 to 60°C) conditions. Researchers often focus on temperature as a crucial parameter in the AD system, particularly during biogas generation. Temperature significantly influences microbial activity, bacterial community structure, process stability, and hydrolysis kinetics within the system [29], [45]–[47]. Bacteria in mesophilic reactors typically grow best between 25°C and 40°C, while thermophilic bacteria thrive at

temperatures between 50°C and 70°C. These temperature ranges are critical for promoting high methanogenic bacterial activity in anaerobic digestion processes [14], [48], [49], [42]. Most anaerobic digesters operate either at mesophilic temperatures, around 35°C, or at thermophilic temperatures, around 55°C (Figure 4) [49], [50]. Thermophilic conditions tend to promote microbial growth, enhance biogas production, and improve digestion efficiency due to the faster specific growth rate of thermophilic bacteria compared to mesophilic bacteria [51]. Thermophilic digestion typically results in higher methane production, accelerated degradation of organic acids, and more effective pathogen eradication. It also yields high-quality residue suitable for use as fertilizer or soil conditioner [29], [36], [52], [53]. Mesophilic conditions offer faster operational rates and higher load-bearing limits [50], while thermophilic systems achieve more efficient sterilization and higher methane production [54], [55]. Additionally, thermophilic digestion aids in the destruction of pathogenic organisms, solid reduction, and improved dewatering in the AD system [36].

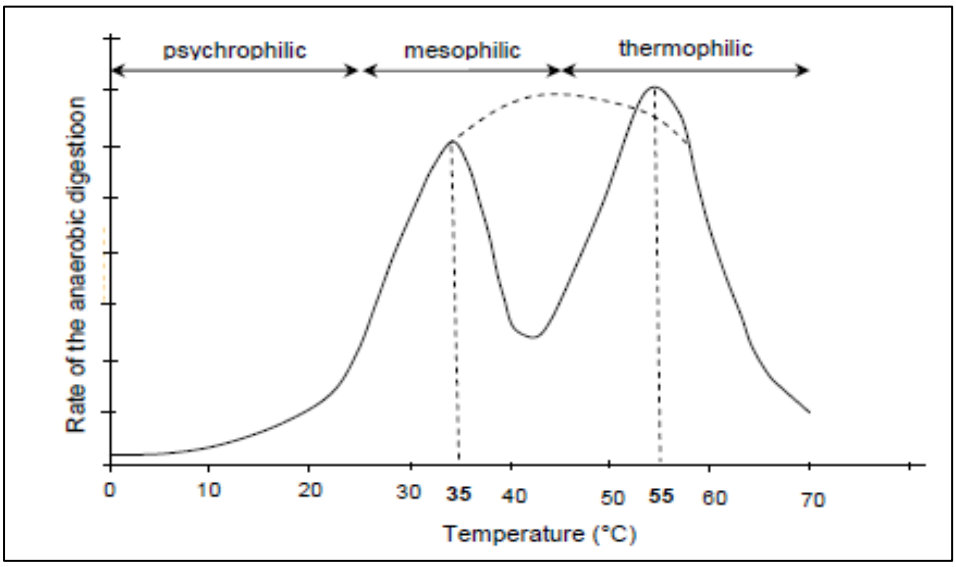


Figure 4. Effect of temperature on the anaerobic digestion process [49], [50]

Retention time in anaerobic digestion refers to the duration that input slurry spends inside the digester [56], [55] and comes in two main types: hydraulic retention time (HRT) and solid retention time (SRT). HRT is the mean duration of slurry inside the digester and is calculated by dividing the volume of the digester by the influent flow rate. On the other hand, SRT is the average time microorganisms spend in the digester. Both HRT and SRT are important factors influencing anaerobic processes and reactor volume [57]. Optimal HRT depends on substrate characteristics, organic loading rate, temperature, and environmental conditions. VFA accumulation can occur with reduced HRT, while prolonged HRT can lead to improper digester component utilization [58]. Studies suggest that longer HRTs are more conducive to methane production, with lower organic loading rates, while shorter HRTs can decrease methane yield. Increasing SRT typically increases HRT and can be achieved by reducing influent flow or utilizing greater digester volume [50]. SRT is considered an important parameter in designing and operating an AD system [36].

Mixing is an important parameter in AD as it significantly influences the behaviour of anaerobic microorganisms [59]. It ensures that anaerobic bacteria receive their nutrients efficiently, prevents scum buildup, and minimizes temperature gradients within the digester [54], [55]. Optimal mixing promotes interaction between substrates and microorganisms, enhancing biogas production. Slow mixing approach is preferred to maintain digester stability and performance [60].

The organic loading rate (OLR) representing the mass of volatile solids (VS) fed into the digester per unit volume per unit time. It impacts methane production, with high rates risking washout of methane and accumulation of inhibitory substances like ammonia and volatile fatty acids (VFA), while low rates result in reduced degradation and methane yield. Optimal OLR balances methane production and economic viability [61], influenced by substrate and operational conditions. Typical ranges vary for low-rate (0.64-1.64) and high-rate (2.40-6.4) digesters [62]. Excessive

substrate addition can destabilize the process, causing VFA accumulation, pH drop, and inhibition of methanogenic bacteria [41]. Short retention times may lead to acidifiers' washout and decreased methane production [63]. Monitoring and adjusting loading rates are essential to prevent process failure [19]. Studies indicate that increasing OLR can enhance biogas production but may reduce methane content [64].

pH plays a vital role in the development of microbes in anaerobic digestion processes. The optimal pH varies depending on the microorganisms present, with hydrolytic bacteria thriving in the pH range of 6-7. Research suggests that controlling pH in a hydrolytic bacteria reactor can enhance microbial degradation by up to twice compared to uncontrolled operations [65]. Figure 5a illustrates how pH control across the range of 5 to 11 influences the solubilization of kitchen waste. Also, rumen organisms demonstrate cellulose degradation at different pH levels (Figure 5b) [66].

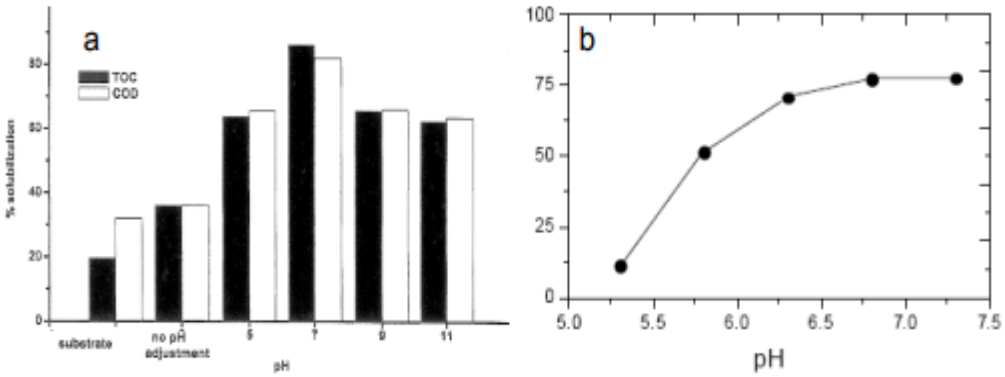


Figure 5. The influence of pH on the degree of anaerobic solubilization [14], [66], [67]

2.4. Inhibition of the anaerobic digestion degradation

In AD systems, inhibition refers to a decrease in the rate of biochemical reactions and bacterial growth, leading to reduced methane output and the accumulation of volatile fatty acids (VFA) [68], [36]. Thermophilic conditions are more prone to inhibition compared to mesophilic conditions. Common inhibitors include ammonia, light metal ions, heavy metals, and various organic compounds. Inhibition levels vary based on factors like feedstock, bacteria, waste composition, methods, and environmental conditions. [66].

Ammonia inhibition poses a significant challenge in anaerobic digestion processes, particularly with complex substrates like organic fraction of municipal solid waste (OFMSW) or manure. Ammonia, a byproduct of nitrogenous compound degradation, accumulates and disrupts the anaerobic digestion food chain. High nitrogen content in substrates such as poultry manure and slaughterhouse waste exacerbate this issue [14], [67]. Chen et al. [69] emphasized the complex mechanism of ammonia inhibition, involving intracellular pH alterations, increased energy maintenance requirements, and enzyme reaction inhibition [70].

Various organic substances, including long-chain fatty acids (LCFA), halogenated aliphatic compounds, and lignin-related compounds, can inhibit anaerobic processes [71], [69]. Toxicity to anaerobic digestion is observed with organic substances like alkyl benzenes and halogenated hydrocarbons [72], [73]. The concentration of these compounds can fluctuate, and their impact on anaerobic processes is influenced by factors such as exposure time, temperature, cell age, feeding pattern, toxicant concentration, and biomass concentration [69].

Light metal ions including Na, K, Mg, Ca, and Al are commonly present in the influent of anaerobic reactors either as chemical adjustments or due to microbial action during biomass breakdown. Heavy metals, unlike light metal ions, cannot degrade naturally and can accumulate to toxic levels, posing a significant threat to anaerobic processes [69].

Volatile fatty acids (VFAs) buildup and pH drop challenge anaerobic digestion. VFAs, like acetate, can hinder methanogens. While VFAs and pH are linked to inhibition, some studies propose VFAs may also hinder organic

matter hydrolysis [74]. However, it's not yet conclusively determined whether VFAs directly inhibit hydrolysis or if it's primarily due to the associated drop in pH [14], [75].

2.5. Biogas technology for the pre-treatment of solid waste

Biogas technology for solid waste pre-treatment has seen significant advancements in reactor design to tackle challenges in anaerobic digestion of biomaterial waste (BMW). These advancements have led to various proprietary systems, making categorization challenging. However, the process is generally classified as "wet" or "dry" [76] based on the solids content in the feedstock or digester slurry. Wet systems typically contain less than 10% total solids (TS) in low solids (LS) configurations, 15%–20% in medium solids (MS), and 22%–40% in high solids (HS) configurations [77]. These systems can be single-phase, where all reactions occur in one digester, or two-phase, where reactors are linked in series [76], [78] - [82].

2.6. Anaerobic degradation system overview

The process of anaerobic degradation of substrate biomass includes the following: Materials sourcing, Source separation of contaminating material, Organic loading rate (OLR), Pre-treatment of the substrate due to digester size, Digestion/degradation of the sample, Methane, CO2, digestate production, and Treatment of the degradation residue.

Pre-treatment is vital for efficient conversion of cellulose in waste biomass to methane, making it more accessible for bacterial action. Cost-effective techniques are needed to avoid toxic by-products and loss of carbohydrates. Through reduction of cellulose crystallinity and polymerization, pre-treatment creates a larger surface area for bacteria, enhancing enzymatic hydrolysis and maximizing biogas production. Pre-treatment methods include physical, chemical, and biological processes [84], offering potential for efficiency enhancement and cost reduction through research and development.

Mechanical particle size reduction techniques include the utilization of Hammer Mills [84], Shear shredders [85], [86], wet pulverisation, Chippers, Grinders, Roll or screw mill, Ball mill and Cutting mills [87], [88], [86], [89], [90].

2.7. Reasons for size reduction in anaerobic digestion processes

Mechanical pre-treatment of organic solid waste involves size reduction, both in the laboratory and on a large scale, for the following reasons:

- Large and bulky materials must be reduced in size before being digested by the anaerobic digestion unit or digester.
- Particle size reduction increases the overall specific surface area of waste particles while decreasing particle size distribution.
- Package (e.g., closed bags) must be accessible.
- Digester stability.

2.8. The effects of various mechanical comminution equipment on feedstock characteristics and particle size distribution

- Mechanical comminution equipment, such as hammer mills and shredders, influences feedstock characteristics and particle size distribution [87].
- Shredding is the most common method for treating mechanical-biological waste, although milling is also utilized [91].
- Particle size distribution (PSD) of solid waste is affected by the type of comminution equipment used [91] - [94].
- Shredders are more energy-efficient and reliable than mills, with a positive overall effect on MSW bioprocessing [95].
- Shear shredders, rotary cutters, and wet macerators produce different particle size distributions, affecting the efficiency of anaerobic digestion [19].

- Particle size reduction techniques, including shredding and crushing, result in varying fractions of organic waste, with fine fractions containing 40 to 90% of the waste [96].
- Hammer mills and ball mills significantly reduce paper and cardboard fractions, with the majority of material ending up in the 40 mm fraction [97].

2.9. Foaming and Causes

In bioprocessing, foaming is a common problem, particularly in agitated and aerated bioreactors. The foam on the sludge surface is defined as a collection of gas bubbles surrounded by a liquid film [102]. Also, foam can occur in many anaerobic digestion digesters, which can have significant effects on the process and result in substantial economic costs. Figure 6; foam images (Abb1) from a biogas digester (Abb2) from a microscope. Foaming causes various problems, such as a reduction in biomethane production and a reduction in organic matter degradation [97]. Low foam concentrations can create a condition that promotes cell damage, potentially leading to more damage. Foaming is triggered by a variety of factors, including:

- Hydrophobic substances, poor mixing, or acetic acid accumulation [98].
- Excess filamentous bacteria such as Gordonia and Microthrix [99].
- Feed sludge composition and inconsistency of digester feed [99].
- Substrates rich in protein and easily degradable [100] .
- Excess surface-active agents example oils and grease [99], [101].
- Unstable conditions caused by shock load or overloading [102], [100].
- Temperature fluctuation [103].
- Air entrainment and solids concentration [99].

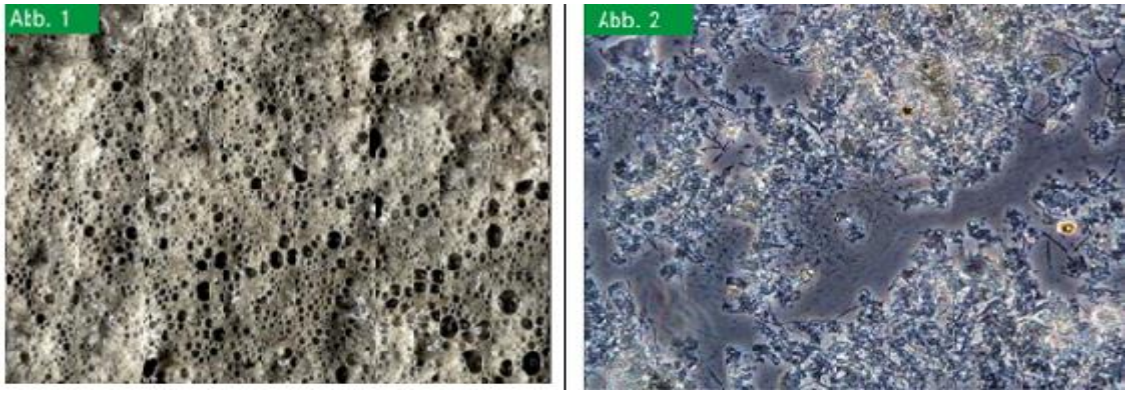


Figure 6. Foam images (Abb1) from a biogas digester (Abb2) from a microscope [104]

The literature review highlights several key findings regarding the impact of pre-treatment and particle size distribution on the performance of anaerobic digestion (AD) systems. It emphasizes the significant influence of pre-treatment, particularly size reduction of anaerobic feedstock particles, on biogas production within AD systems. Particle size distribution plays an important role in AD system performance, with factors beyond mean particle size affecting methane production. Changes in biogas generation due to different particle size distributions may not be significant across all substrates, maintaining a balance in particle size distribution and organic loading is essential to prevent system failure from acidification. Various operational factors can influence observed increases or changes in biogas yield resulting from substrate pre-treatment, emphasizing the complexity of optimizing AD system performance. Further research is required in these areas, case studies could provide valuable contributions to the existing body of knowledge. Therefore, it is vital to explore the various particle sizes distribution of the substrate fed into the digester to have a better understanding of the particle size of the feedstock and how they affect the efficacy of the AD process.

The rest of this paper is structured as follows: Section 3 outlines the experimental design and details the materials and methods used in this study. Section 4 presents and analyzes the experimental results. Section 5 summarizes the conclusions drawn from this work and suggest areas for further research.

3. Materials and Methods

3.1. Feedstock and Inoculum

Four waste biomasses are used in this investigation, namely (i) paper waste was collected from the University of Sheffield Energy Group Offices. (ii) Banana peel is collected from households (iii) grasses are obtained from the University of Sheffield and tomato waste obtained from the moor market in Sheffield. The feedstock is source-separated from three selected routes. After a sufficient sample has been collected (20kg), the raw waste is screened to remove any contaminants (if any), and then homogenised for characterisation and further size reduction. Also, anaerobic fresh active digestate was also collected from the existing mesophilic AD energy plant at Blackburn Meadows (BbM) wastewater treatment works (WwTW). Before using fresh active digestate from mesophilic digesters, the active digestate was filtered with a 1mm mesh sieve to remove 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, a quantity of the respective waste feedstock was measured and divided into four (4) equal parts. Each biomass type is subject to four particle size methods which begin with a coarse chopping/shredding operation (PTL1), a finer chopping operation (PTL2) and a maceration/mincing operation (PTL3), process is shown in figure 7 and table 1 which depicts the nomenclature for each biomass size reduction, with differences in pre-treatment levels, including processing time, and reduction mechanisms. However, pre-treatment 2 (PTL2) involved passing 3/4 of the waste through a Mincer Ring RAUT 12 16#, for 2 minutes, pre-treatment level 3 (PLT 3) involved passing the 2/3 parts of the waste through a food processor with a cut 5200 (Grinder for 3 minutes). Pre-treatment level 4 (PTL4) involved 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 5^0C before the experiment. Each fraction is characterised by its particle size distribution by the most relevant method. However, the generation of biogas yield depends upon the type of feedstock utilized.

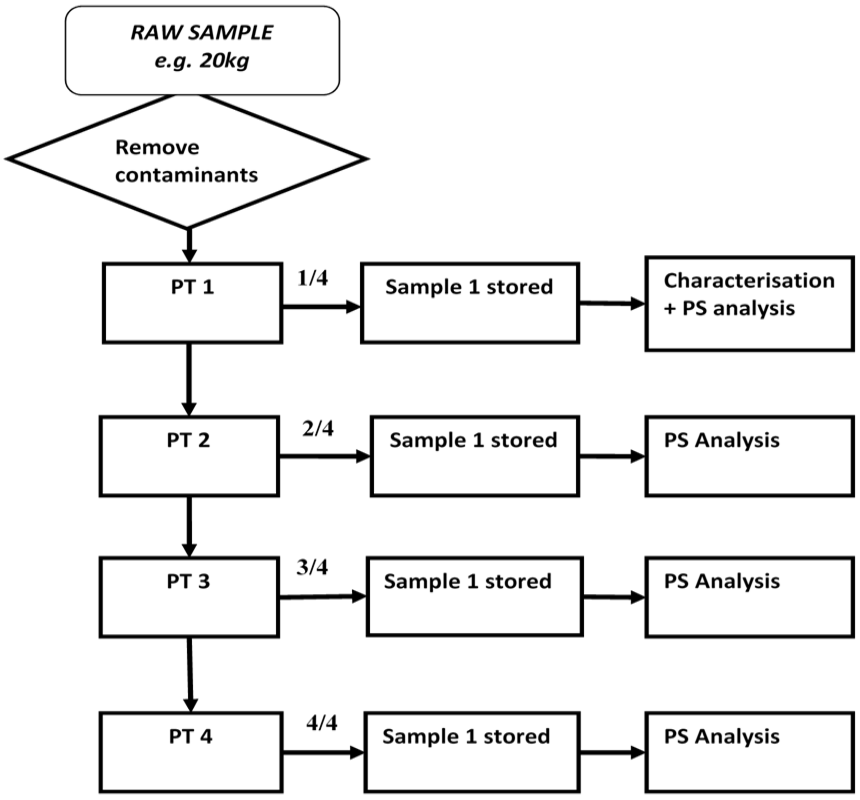


Figure 7. Schematic of the sample preparation methodology

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 shows the parameters examined in this study for feedstock physicochemical and biological composition, such as total solids (TS) and volatile solids (VS), pH, alkalinity, and elementary analysis, biogas composition, and volume. The total solid (TS) and volatile solid (VS) contents of the digester's liquid digestate were determined. pH and alkalinity analysis were used to determine the digestate's stability. Methane and CO_2 levels in biogas were measured. The study's reagents were purchased from the fisher Scientific" (Loughborough United Kingdom). The chemical is 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+ [105] 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) [105]. 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 [105]. 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 [106], 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 [14]

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 [107]. Optimal methane production demands a carbon-to-nitrogen ratio of 25:1. The Buswell equation [108] enables the calculation of water usage and methane and carbon dioxide production when a known mass of volatile solid (VS) undergoes anaerobic digestion. [108]:

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

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) [109]–[112]. Studies have shown that determining the CV based on elemental composition or ultimate analysis yields the most accurate and precise results [112], [113]. 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^{o}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 (Figure 8). 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^{0}C. The gas volume measuring device was flushed with methane calibration gas at 5l/min for 60 seconds to create the anaerobic condition.

Figure 8. Leak testing of batch reactor

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. The experimental setups for BMP testing are depicted in figure 9.

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

Where:

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

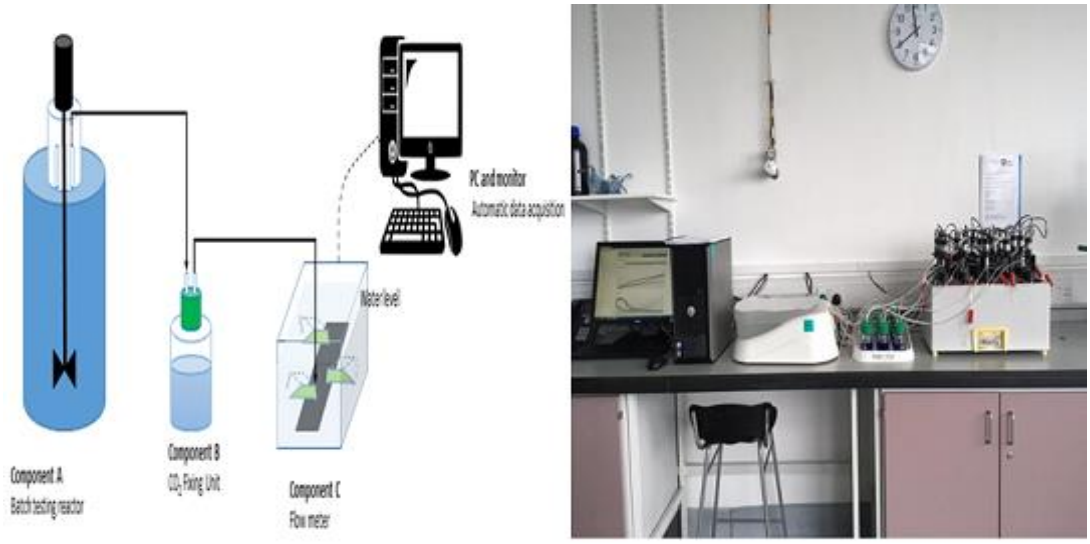


Figure 9. Experimental setups for BMP testing, stirred incubation unit in a water bath with NaOH as a scrubber and a volumetric gas flow meter multi-channel and software user interface

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. The experimental setup is depicted in Figure 10.

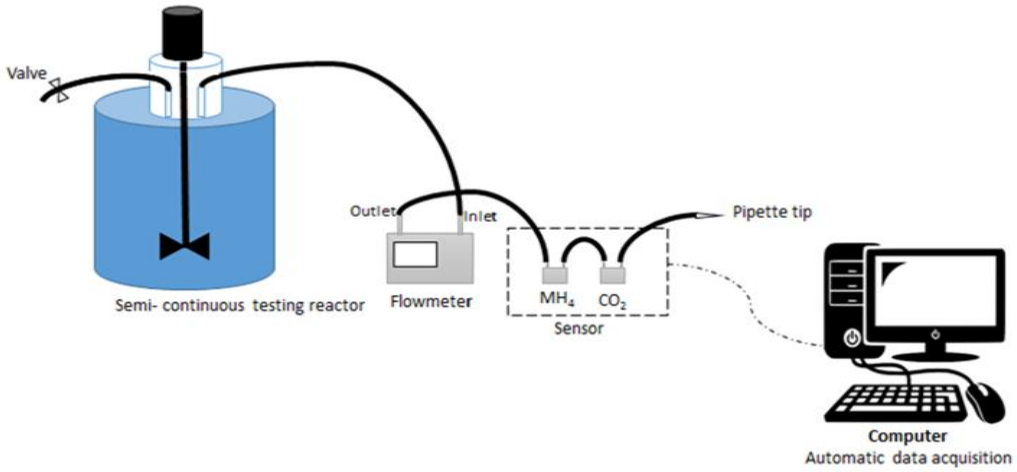


Figure 10. Experimental setups for semi continuous testing

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).

3.10. Overview of experimental set-up

Figure 11 presents a comprehensive depiction of the experimental setup, offering insights into the methodology utilized throughout the study. It serves as a visual guide to understand the procedures and techniques employed in the research.

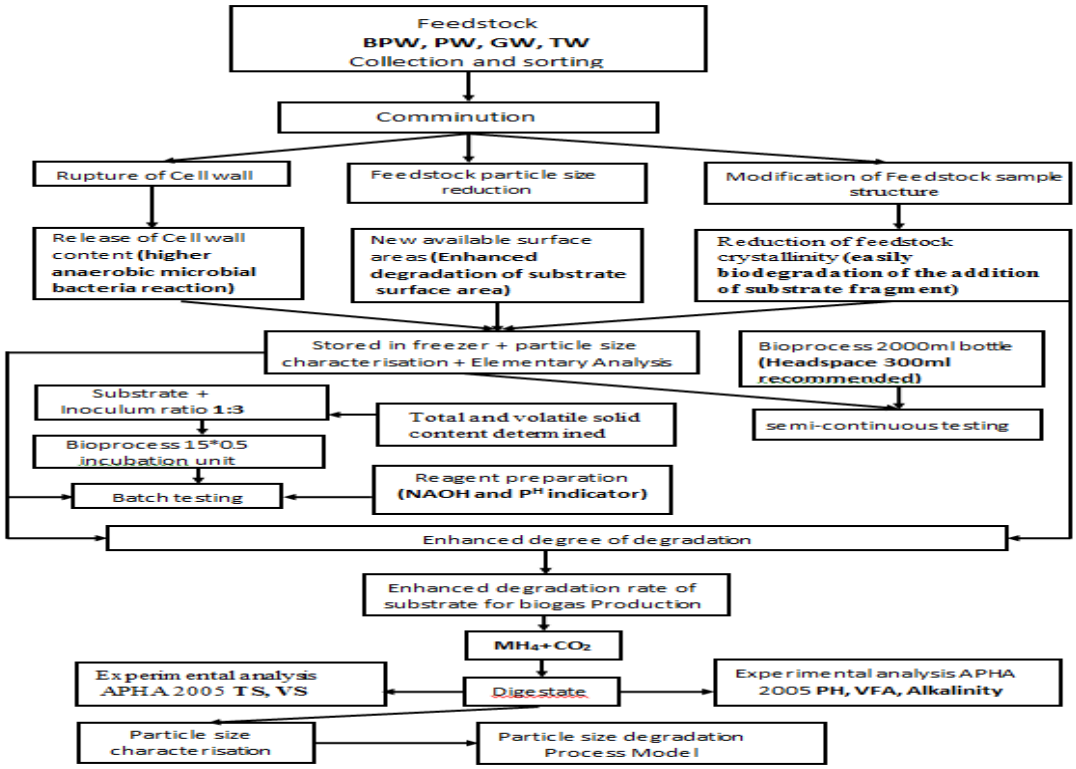


Figure 11. Overview methodology of batch and semi-continuous testing

3.11. Particle size distribution determination

A representative sample of well-mixed substrate biomass (SBS) was dried, followed by imaging using a camera across different levels of pre-treatment (PTLs 1-4). ImageJ particle size analyzer was employed to analyze the images, with each test marked according to the sample and its pre-treatment level. Approximately 4g of each sample per PTL was analyzed. During processing, a background colour was chosen to enhance contrast for better visualization. ImageJ was calibrated to convert pixel representation to physical units (mm) for accurate particle size measurement [114], [115]. Initially, images were converted to grayscale and then binarized for analysis using the ImageJ particle size analyzer plugin. The analytical procedure is depicted in figure 12. Graphs of size distributions were plotted using Origin-pro software 2022.

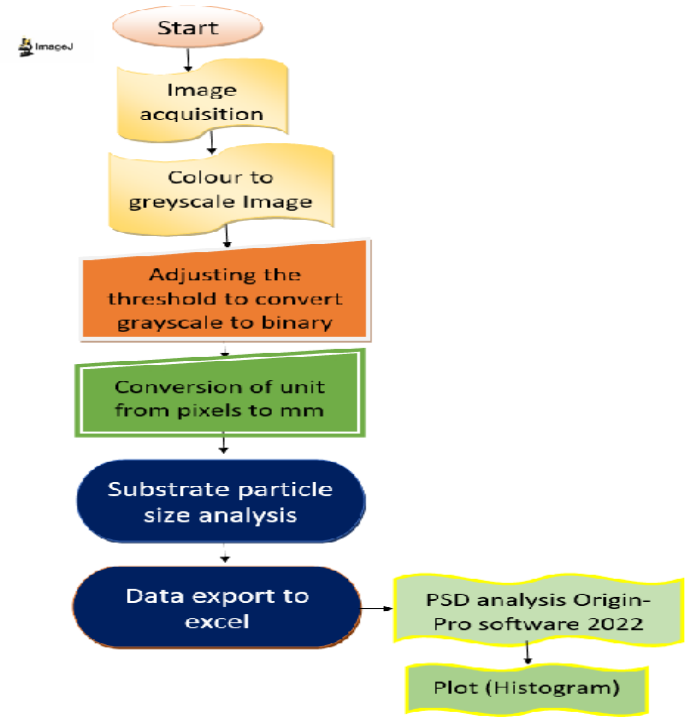


Figure 12. Process flow for characterising particle size distribution

4. Results and discussion

4.1. Banana waste particle size distribution

The particle size distribution (PSD) results for Banana Peel Waste Substrate Biomass (BPWSB) across four pre-treatment levels (PTLs) are presented in Figures 13 to 16, with histograms depicting normal distribution curves. Mean values and standard deviations are reported in Table 6, while photographs of each pre-treatment level are provided in appendix A1. The particle sizes varied across PTLs, with PTL 1 ranging from 0 to 14.5mm, followed by PTL 2 (0 to 8.5mm), PTL 3 (0 to 5.25mm), and PTL 4 (0 to 2.1mm). In PTL 1, 40% of the particles ranged from 6 to 8mm, 25% were between 3 to 6 mm, 16% between 8 to 10mm, <2mm diameter constituted 14%, and <5% fell within the 10 to 15mm range. For PTL 2, 60% of particles were <3mm, with 40% ranging from 3 to 8.5mm. Similarly, PTL 3 had approximately 72% of particles between 0.5 to 2.5 mm, while in PTL 4, 87% of particles were between 0.1 to 0.6mm, with the remaining 13% falling within 0.65 to 2.1mm. Smaller particles (<2mm) were predominant in PTLs 2, 3, and 4, enhancing biodegradability and methane production rates. Pre-treatment into smaller particles increases the feedstock surface area, favouring the anaerobic digestion (AD) process. However, the irregular shape and texture of wet materials pose challenges in accurately classifying size distributions, as mechanical breakdown alters the sample's structure and distribution profile, potentially impacting biodegradability.

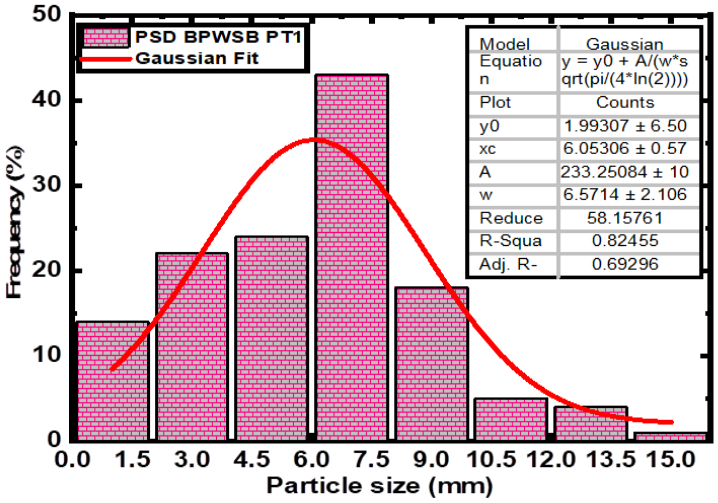


Figure 13. Particle size distribution of banana waste substrate biomass pre-treatment 1

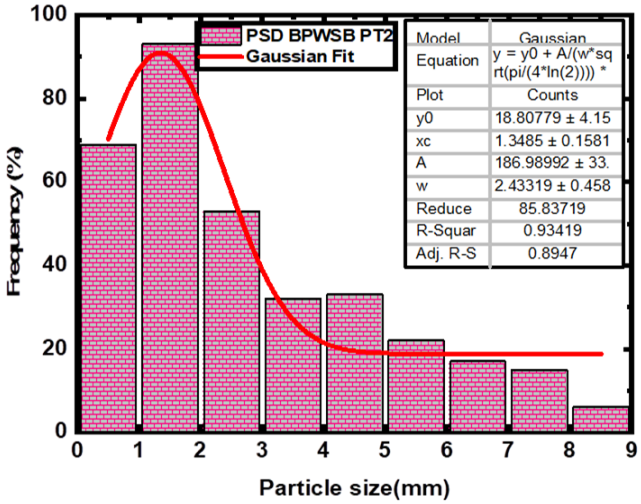


Figure 14. Particle distribution of banana peel waste substrate biomass pre-treatment 2

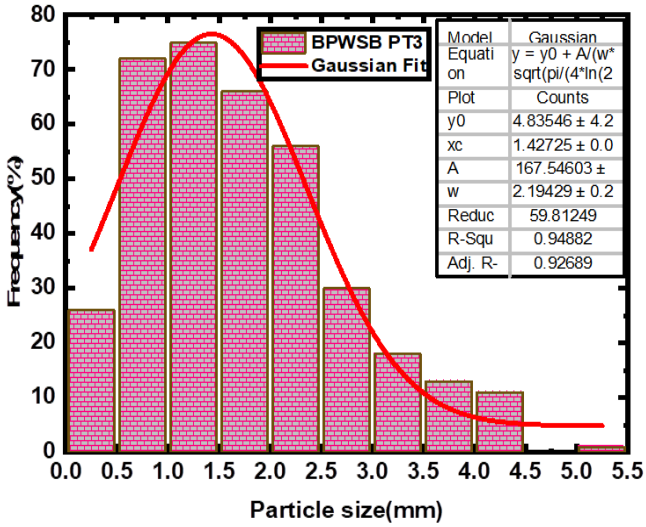


Figure 15. Particle size distribution of banana peel waste substrate biomass pre-treatment

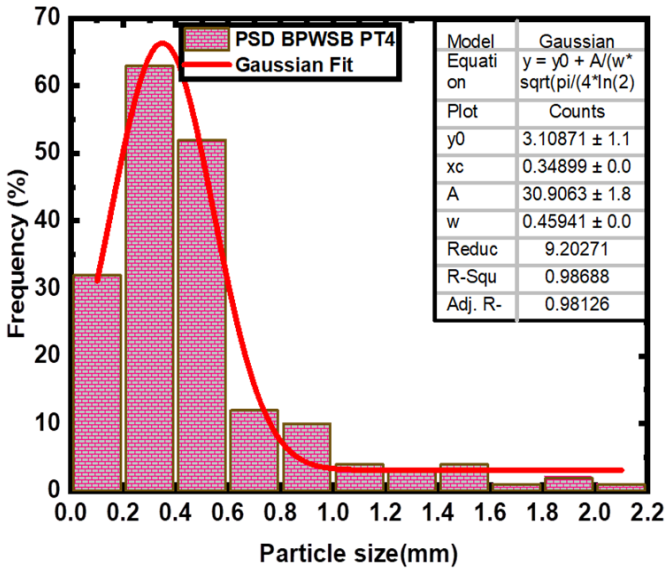


Figure 16. Particle size distribution of banana peel waste substrate biomass pre-treatment four

Table 6. Summary of the particle size distribution of the banana waste substrate biomass according to the pre-treatment levels used during experimental testing of batch and semi-continuous testing

Substrate identity, pre-treatment level and size distribution				
Substrate biomass and Pre-treatment level	PSD (mm)	Mean PSD (mm)	PSD	Av. PS with error
Banana peel waste substrate biomass				
BPWSB PT1	1-15	6.05	(∂) = w/2=3.29	XC±∂= 6.05±3.29
BPWSB PT2	0.5-8.5	1.35	(∂) = w/2=1.22	XC±∂= 1.35±1.22
BPWSB PT3	0.25-5.25	1.43	(∂) = w/2=1.095	XC±∂= 1.43±1.095
BPWSB PT4	0.1-2.1	0.35	(∂) = w/2=0.23	XC±∂= 0.35±0.23

4.2. Grass waste particle size distribution

The particle size distribution (PSD) results for Grass Waste Substrate Biomass (GWSB) across its four pre-treatment levels (PTLs) are depicted in histograms with normal distribution curves in Figures 17 to 20. Mean values and standard deviations are provided in table 7, and a photograph of the GWSB is included in Appendix A2. The pre-treatment levels of GWSB, determined by grinding processes, resulted in varied particle size ranges: PTL 1 ranged from 3 to 17mm, PTL 2 from 0.5 to 9.5mm, PTL 3 from 0.5 to 7.5mm, and PTL 4 from 0.25 to 4mm. Predominantly, PTL 1 comprised 90% of particles >3mm, while PTL 2 had 56% of particles <3mm, and both PTL 3 and PTL 4 contained 62% and 90% of particles <3mm, respectively. The abundance of finer particles in PTLs 2, 3, and 4 suggests they favour the metabolic process, contrasting with the larger particles prevalent in PTL 1. Grass Waste Substrate Biomass (GWSB) is indicated to have minimal adverse effects on the bioreactor due to its reduced coarse material content, despite grass digesters often encountering issues with particle floating and matting [116] - [117].

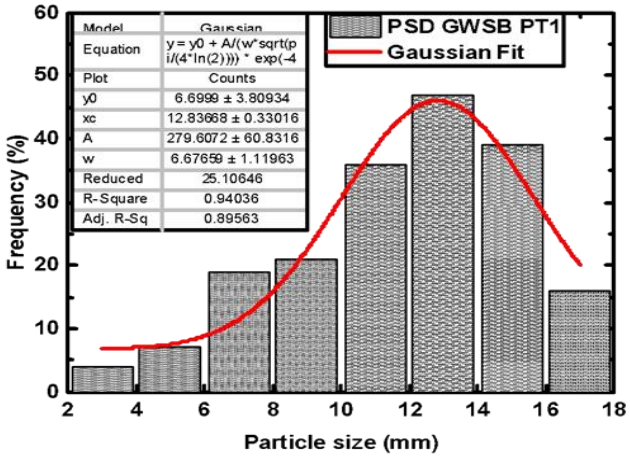


Figure 17. Particle size distribution of grass waste substrate biomass pre-treatment level 1

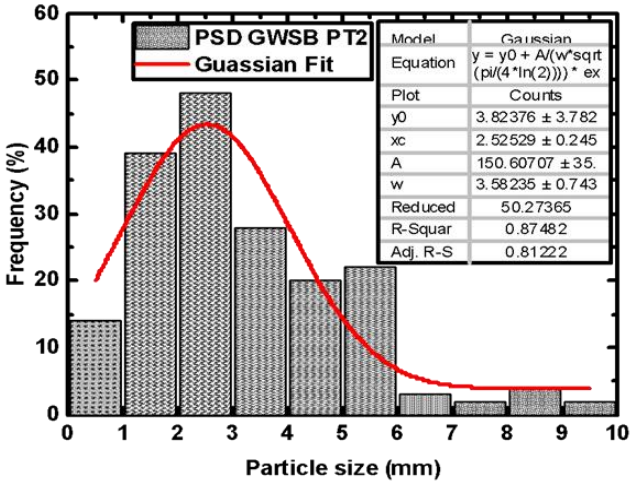


Figure 18. Particle size distribution of grass waste substrate biomass pre-treatment level 2

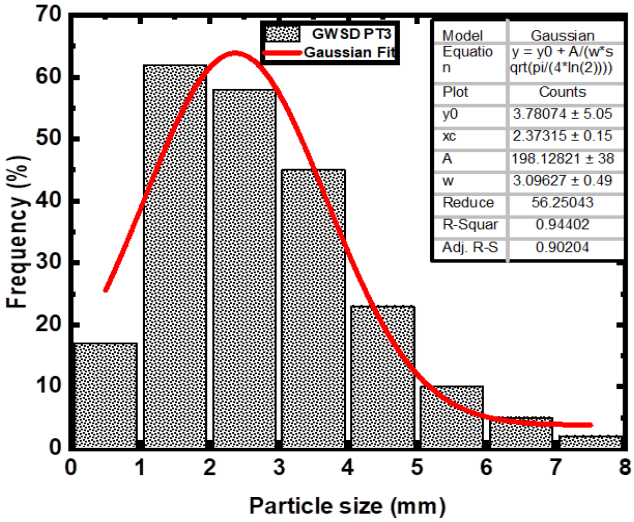


Figure 19. Particle size distribution of grass waste substrate biomass pre-treatment level 3

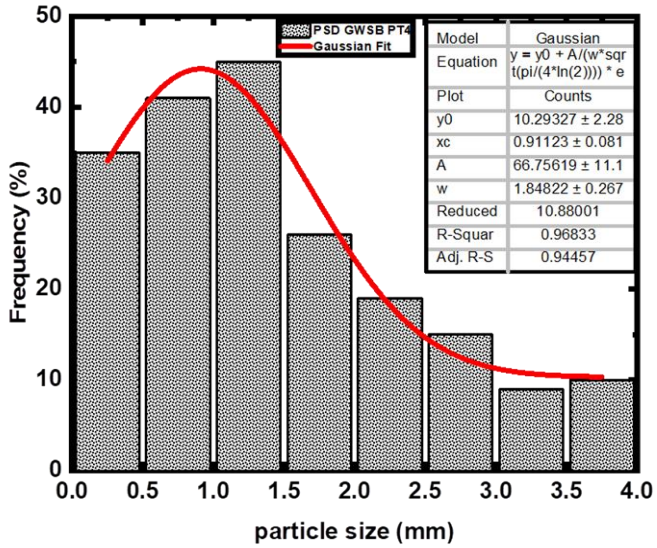


Figure 20. Particle size distribution of grass waste substrate biomass pre-treatment level 4

Table 7. Summary of the particle size distribution of the grass waste substrate biomass according to their pre-treatment level used during experimental testing of batch and continuous testing

Substrate identity, pre-treatment level and size distribution				
Substrate biomass and Pre-treatment level	PSD (mm)	Mean PSD (mm)	SD	Av. PS with error
Paper waste substrate biomass				
GWSB PT1	1-17	12.84	(∂) = w/2=3.34	XC±∂= 12.84±3.34
GWSB PT2	0.5-9.5	2.53	(∂) = w/2=1.79	XC±∂= 2.53±1.79
GWSB PT3	0.5-10.5	2.37	(∂) = w/2=1.55	XC±∂= 2.37±1.55
GWSB PT4	0.1-2.1	0.91	(∂) = w/2=0.92	XC±∂= 0.91±0.92

4.3. Paper waste particle size distribution

The Particle Size Distribution (PSD) results for Paper Waste Substrate Biomass (PWSB) are displayed in Figures 21 to 24, representing four pre-treatment levels (PTLs). PTL 1 involves shredding with water addition, while PTLs 2, 3, and 4 undergo further grinding. The histograms with normal log distribution curves, mean values, and standard deviations are detailed in Table 8, accompanied by photographs in Appendix A3. Across the PTLs, particle size ranges vary: 1-25mm for PTL 1, 0.5-14.5mm for PTL 2, 0.5-10.5mm for PTL 3, and 0.25-4.75mm for PTL 4. Particularly, PTL 4 exhibits a significant proportion (85%) of smaller particles <3mm, contrasting with PTLs 1-3. This trend aligns with the preferable particle size for anaerobic digestion (AD) processes. Despite PWSB containing more significant particles in PTLs 1-3, an increase in finer particle content in PTL 4 is observed, likely influenced by its physicochemical properties, particularly lignin. The prevalence of smaller particles in PTL 4 reflects their suitability for AD processes, addressing historical issues of foaming and clogging faced by paper digesters.

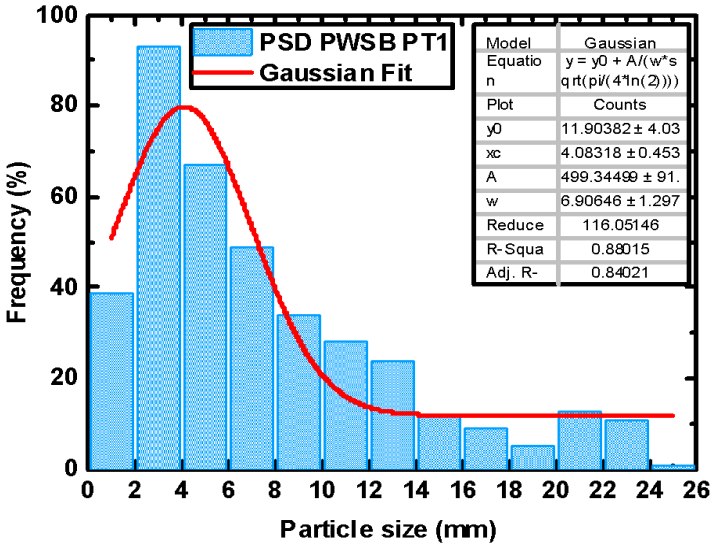


Figure 21. Particle size distribution of paper waste substrate biomass pretreatment 1

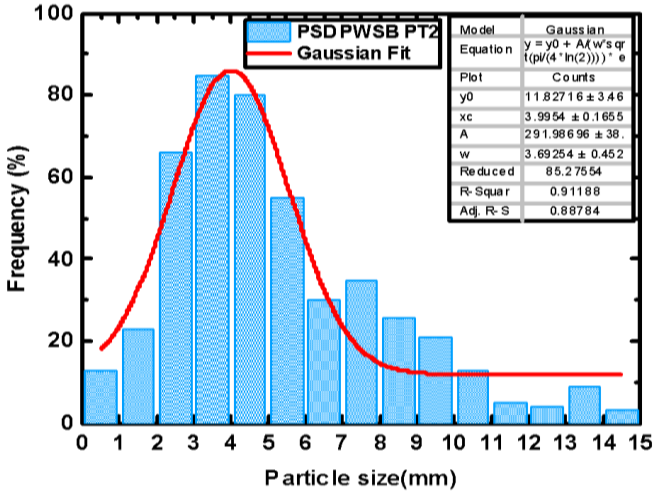


Figure 22. Particle size distribution of paper waste substrate biomass Pretreatment 2

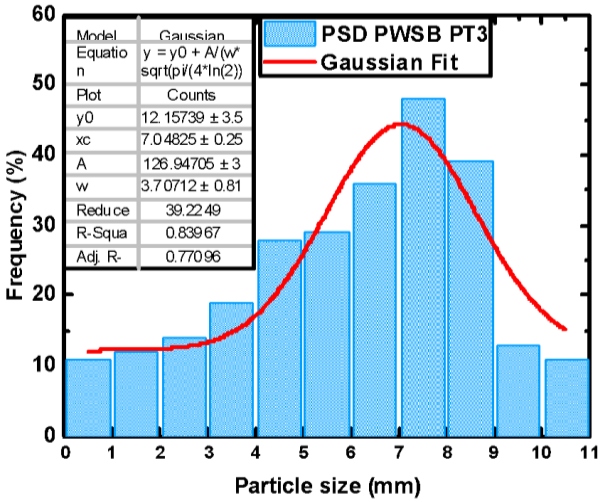


Figure 23. Particle size distribution of paper waste substrate biomass pretreatment level 3

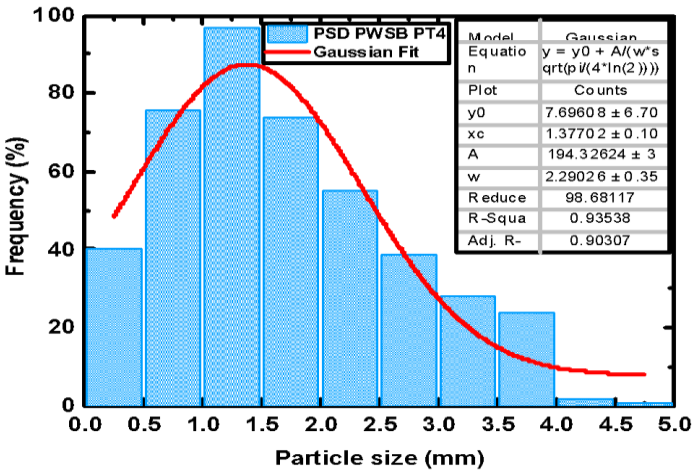


Figure 24. Particle size distribution of paper waste substrate biomass pretreatment level 4

Table 8. Summary of the Particle size distribution of the paper waste substrate biomass according to their pre-treatment level used during experimental testing of batch and continuous testing

Substrate identity, pre-treatment level, size distribution				
Substrate biomass and pre-treatment level	PSD (mm)	Mean PSD (mm)	SD	Av. PS with error
paper waste substrate biomass				
PWSB PT1	1-25	4.08	(∂) = w/2=3.46	XC±∂= 4.08±3.46
PWSB PT2	0.5-14.5	3.99	(∂) = w/2=1.85	XC±∂= 3.99±1.85
PWSB PT3	0.5-10.5	7.05	(∂) = w/2=1.86	XC±∂= 7.05±1.85
PWSB PT4	0.25-4.75	1.38	(∂) = w/2=1.15	XC±∂= 1.38±1.15

4.4. Tomato waste particle size distribution

The last sample used is TWSB, and the PSD results for its four PTLs are presented as histograms with a curve of the normal distribution as successively shown in Figs. 25 to 28. Its mean PSD value and SD are also detailed in table 9. The photograph of the TWSB is shown in appendix A4. The four pre-treatment levels of different particle sizes of the TWSB are also the result of the maceration/grinding process. PTL 1 ranged from 1 to 11mm, PTL 2 (0.75 to 7.25mm), PTL 3 (0.25 to 3.75) and PTL 4 (0.1 to 1.5mm). Considering PS abundance, PTL 1 has 41% of particles < 3mm, PTL 2 has 66% of particles < 2.9mm, PTL 3 contains 94% of particles < 3mm while PTL 4 entirely contains finer particles between 0.1 and 1.5mm. Amongst all the PTLs of TWSB, particles of PTL4 favoured the AD process better due to their sizes which were much smaller in diameter and agrees with general standards.

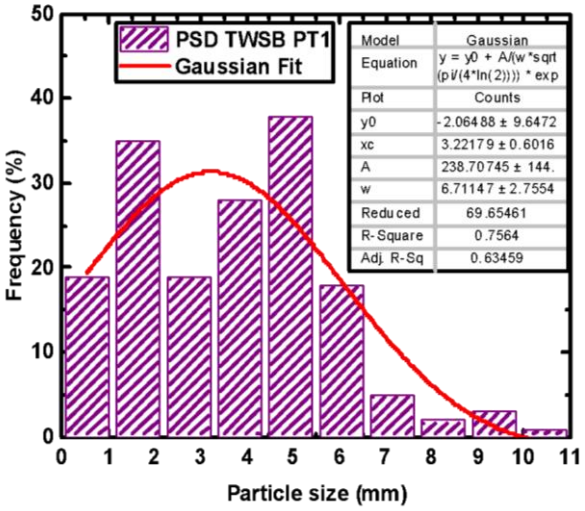


Figure 25. Particle size distribution of tomato waste substrate biomass pretreatment level 1

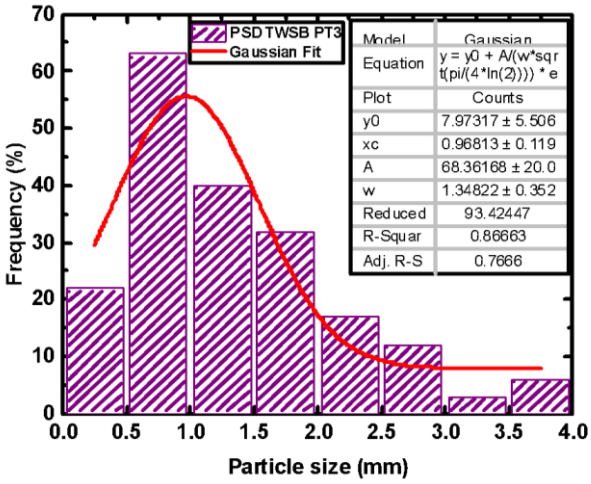


Figure 26. Particle size distribution of tomato waste substrate biomass pretreatment level 2

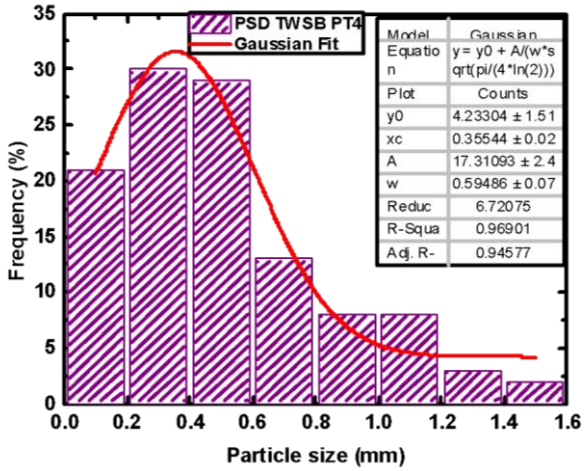


Figure 27. Particle size distribution of tomato waste substrate biomass pretreatment level 3

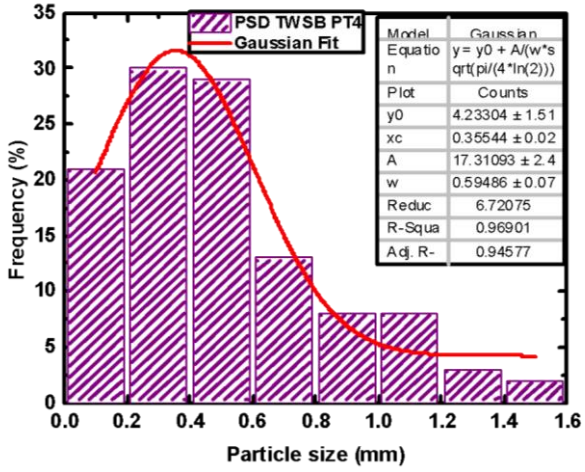


Figure 28. Particle size distribution of tomato waste substrate biomass pre-treatment level 4

Table 9. Summary of the Particle size distribution of the tomato waste substrate biomass according to their pre-treatment level used during experimental testing of batch and semi continuous testing

Substrate identity, pre-treatment level and size distribution				
Substrate biomass and Pre-treatment level	PSD (mm)	Mean PSD (mm)	SD	Av. PS with error
Tomato waste substrate biomass				
TWSB PT1	1-11	3.22	(∂) = w/2=3.36	XC±∂= 3.22±3.36
TWSB PT2	0.75-7.25	2.05	(∂) = w/2=1.41	XC±∂= 2.05±1.41
TWSB PT3	0.25-3.75	0.97	(∂) = w/2=0.68	XC±∂= 0.97±0.68
TWSB PT4	0.1-1.5	0.36	(∂) = w/2=0.30	XC±∂= 0.36±0.30

4.5. Effects of various mechanical devices on methane yield in biogas

The effects of different mechanical equipment on the production of biogas methane were studied. This study used four different substrates broken down into four PTLs with increasing processing time of 2mins (PTL2) 3mins (PTL3) and 5mins (PTL4). As was previously noted, the substrate's PS size significantly affects how quickly it degrades [117].

The methane output of different particle sizes produced by various mechanical equipment followed a similar pattern: the higher the degradation rate, as a result, the higher the methane output. The methane potential for PTL1 of the non-treated GWSB and TWSB, manually chopped (BPWSB), and shredded (PWSB) feedstocks was tested. The manual chopping of BPWSB coarse material (PTL1) produced a methane production that was roughly 21%, 6.1%, and 5.2 more than that of GWSB, PWSB, and TWSB.

The next best performing material was TWSB (non-treated), which outperformed PWSB and GWSB in terms of methane yield by 3.1% and 1%, respectively, while exceeding GWSB by 13.8% in terms of PWSB that had been shred. The biomass from the untreated GWSB produce the least methane. The measured methane output of the BPWSB was found to be much higher at roughly 20%, 8.4%, and 4.2% when compared to the GWSB, PWSB, and TWSB. This resulted from the maceration/mincing technique used to treat PTL2. The TWSB trailed closely after,

outperforming the GWSB and PWSB by nearly 15% and 4%, respectively, while the PWSB exceeded the GWSB by 10.6%.

The GWSB produce the least amount of methane. The BPWSB also produced significantly more methane than the GWSB, PWSB, and TWSB, at a rate of about 16%, 11% and 5%. This resulted from the grinding process used in PTL3 treatment. The TWSB outperformed the GWSB and PWSB by roughly 9.1% and 5%, respectively, whereas the PWSB outperformed the GWSB by 4.3%.

Methane production is lowest in the biomass from the GWSB. Similarly, compared to the GWSB, PWSB, and TWSB, the BPWSB produced significantly more methane, at roughly 21%, 21% and 13%. This resulted from the processing of PTL4 using the combination mincer and grinder methods. The PWSB and GWSB produced the lowest amount of methane. Compared to BPWSB and TWSB, the methane output from TWSB is almost 7.3% higher. This was due to the pre-treatment method's capacity to effectively decrease the material while also destroying the substrate. The size reduction caused an increase in methane production [118], [117]. The least efficient pre-treatment method also led to a rise in biogas production. When the results of the laboratory batch tests are compared, the mechanical pre-treatment methods, which uses a combination of a mincer and a grinder to treat PTL4, produces more methane and results in a higher volatile solid reduction (VSR), as opposed to the grinder and mincer used to treat PTL3 and PTL2, as well as the course/chopping/shredding and untreated material (PTL1).

A grinder that was used to treat PTL3 material exhibited higher methane output than a mincer that was used to treat PTL2 material. The output of methane from coarse materials did not substantially increase. This is a result of the materials' size. Though, BPWSB that has been manually chopped outperforms the other three substrates in terms of methane yield, followed by TWSB that hasn't been treated, while PWSB that has been shred and non-treated GWSB have much less of an impact on the amount of methane output. When the methane yields for the various substrates were compared, it was discovered that BPWSB and TWSB performed best on average.

The lowest amount of methane is produced in GWSB. The average methane production efficiencies for the BPWSB, GWSB, PWSB, and TWSB are shown in table 10. The performances of the substrates at different pre-treatment degrees were compared. The results show that the PWSB did best at Pre-treatment level 1-3, while the BPWSB and TWSB did better overall at pre-treatment level 1-4. Although PTL4 of GWSB and PWSB were identical, GWSB produced the least amount of methane among PTL1-3.

Table 10. Measured and predicted maximum specific methane production with respective % volatile solids reduction

Parameter	BMP experimental	BMP theoretical	% VSR
PTL1	245	321	60.4
PTL2	271	327	61.9
PTL3	293	339	63.5
PTL4	332	353	66.7
AV	285.2	353	63.4

4.6. Comparing the responses of the chosen substrates to various treatments in terms of methane yield and volatile solid reduction

Table 11 and figure 29 compares the four selected substrates, their responses to the various treatments, and the amount of methane produced. It reveals that the yields of the untreated TWSB were similar to those of the PWSB that had been shred. The chopped BPWSB were significantly higher compared to those of untreated TWSB containing more smaller PS, GWSB, and shredded PWSB. Untreated TWSB produced significantly more methane

than untreated GWSB, while chopped BPWSB produced significantly more methane than shredded PWSB. This could be attribute to substrates' lignin content, which inhibits anaerobic digestion [119] - [121]. The results from the shredded PWSB are like those from [122], [123] which showed that wastepaper yielded 210 - 217 Nml/gVS, though methane yield was slightly higher as shown in table 11 for PTL1. While the result of the minced treatment (PTL2) of TWSB was slightly higher than the PWSB, GWSB produced the least amount of methane, the output of the minced BPWSB was greater than that of the TWSB, GWSB, and PWSB. The rate of biogas production from minced banana peel was slightly higher than chopped banana peel, and the combined effect of the grinder and mincer was significantly greater than others three PTLs. Although the banana peel that had been ground was much higher than the banana peel that had been chopped and minced. A similar pattern was seen with grind treatment (PTL3), as well as the combined impact of the minced and grinded treatments, as shown in table 11 (PTL4) for all substrates. The combined effects of grinding and mincing showed a quick response to the rate of degradability and higher VSR because they produce particles with a greater surface area than the other three treatment methods. These were followed by ground, minced, and untreated TWSB, chopped BPWSB, untreated GWSB, and shredded PWSB, which initially did take a little longer to degrade due to its lesser surface area.

4.6.1 Biochemical Methane Potential Kinetics of Substrate Biomass

Appendix A5-1 presents the results of the Biochemical Methane Potential (BMP) kinetic model for banana peel waste substrate biomass (BPWSB). The analysis used the modified Gompertz model and first-order kinetics, with SPSS for statistical analysis. First-order kinetics provided a better fit for pre-treatment levels 1-4, with higher R^2 values (0.965, 0.944, 0.976, and 0.959) compared to the modified Gompertz model (0.937, 0.915, 0.952, and 0.929). The modified Gompertz model fit better for cellulose with an R^2 of 0.965. PTL4 had the highest k-value (0.59) for first-order kinetics due to its large surface area and the presence of easily assimilated microbial fermentation products.

The results of the Biochemical Methane Potential (BMP) kinetic model for grass waste substrate biomass (GWSB) are presented Appendix A5-2. First-order kinetics and the modified Gompertz model were used to derive parameter values. First-order kinetics showed a better fit for pre-treatment levels 1-4 and cellulose, with R^2 values of 0.965, 0.994, 0.976, 0.942, and 0.922. PTL3 had the highest k-value (0.339) for first-order kinetics, and its K-value was slightly higher than that of PTL4.

Appendix A5-3 presents the results of the Biochemical Methane Potential (BMP) kinetic model for paper waste substrate biomass (PWSB). First-order kinetics provided a better fit compared to the modified Gompertz model, with consistent R^2 values of 0.956 for all pre-treatment levels (PTLs). PTL4 had a higher k-value (0.59) due to its large surface area. Similar to the results for BPWSB and GWSB, PTL3 had a lower k-value than PTL4, followed by PTL2 and PTL1. The model also indicated a short lag phase before bacterial utilization of the substrate for all four PTLs.

The results of the Biochemical Methane Potential (BMP) kinetic model for tomato waste substrate biomass (TWSB) are presented in Appendix A5-4. Both the modified Gompertz model and first-order kinetics were used to obtain parameters. The first-order BMP kinetics provided a better fit compared to the modified Gompertz model. PTL4 had the highest k-value (0.59) due to its large surface area, similar to the results for BPWSB, GWSB, and PWSB. PTL1's k-value was higher than PTL2's, while PTL3's k-value was lower than PTL4's.

Table 11. Comparison of the methane yield of the four-substrate biomass in Nml/gVS

Substrate biomass	Treatment	PTLs	CH_4 yield Nml/gVS	% Increase	VSR	% Increase	CH_4 yield (literature data)	Reference
BPWSB	Manual Chopped	1	266.6	-	60.4		77-336 Nml/gVS	[124], [125] – [130]
	Mincing	2	293.9	10.2	61.9	0.6		
	Grinding	3	318.9	19.6	63.50	1.2		

	Mincing + grinding	4	334.2	25.4	66.70	2.4		
	AV		303.4	-	63.4			
GWSB	As Collected	1	201.9	-	52.5		117-467 Nml/gVS	[124], [131]–[134], [119], [135],
	Mincing	2	224.5	11.2	53.4	0.4		
	Grinding	3	253.8	25.7	57.4	2.4		
	Mincing + grinding	4	270.3	33.9	59.1	3.3		
	AV		237.6		55.2			
PWSB	Shredded	1	231.0		52.4		107-369 Nml/gVS	[135], [125], [122]
	Mincing	2	250.1	8.3	53.3	0.4		
	Grinding	3	264.8	14.6	55.0	1.1		
	Mincing + grinding	4	273.8	18.5	56.0	1.6		
	AV		254.9		54.2			
TWSB	As Collected	1	233.1	-	57.9		199-384 Nml/gVS	[136], [129]
	Mincing	2	260.2	11.6	60.8	1.2		
	Grinding	3	276.4	18.6	62.2	1.8		
	Mincing + grinding	4	294.2	26.2	63.0	2.2		
	AV		266.0		61.0			

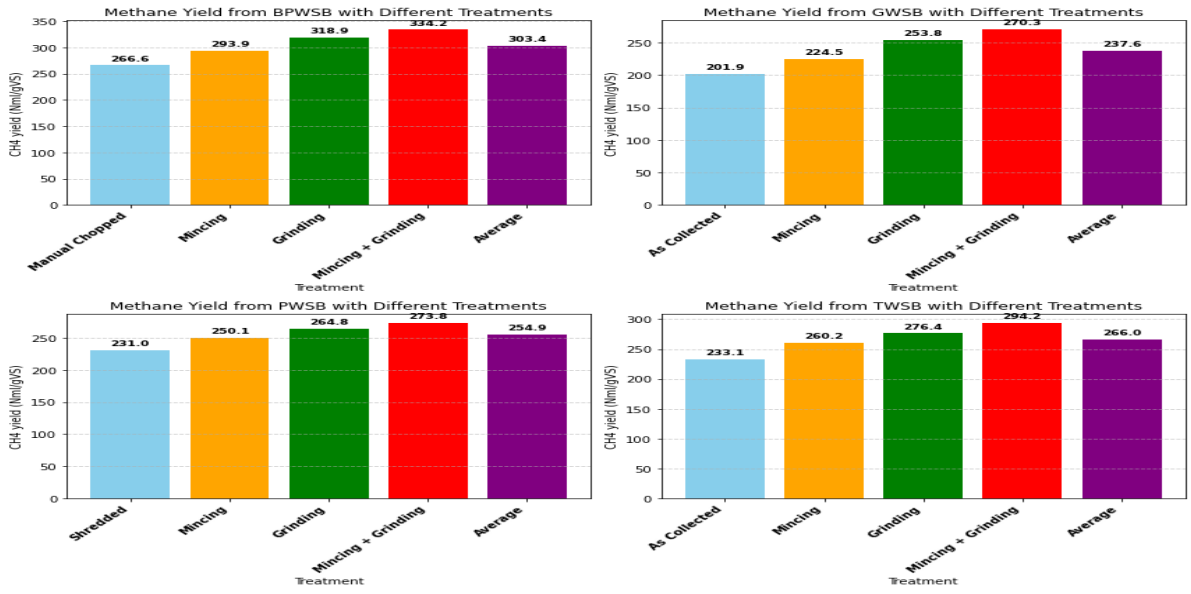


Figure 29. Effect of treatment methods on methane yield across various substrate biomass types

5. Conclusions

At a laboratory scale, PSD was tested in the output of mechanical size reduction equipment for four distinct substrates: BPWSB, GWSB, PWSB, and TWSB. Although large PS were more predominant in the distribution of some substrates that were processed using the same mechanical method and for the same amount of time as others, the shift in PSD distributions profile is the most obvious difference. The following conclusions were drawn from this study, which involved mechanical pre-treatment of the substrate with a various size reduction equipment and operation modes:

- Tomato waste from Sheffield's moor market was found to include a significant number of small size particles without being mechanically changed or pre-treated in PTL1 as compared to grass waste from the University of Sheffield, PWSB was collected from the University of Sheffield Energy Group Offices which was shredded, and banana peel collected from households was found to be presents after being manually chopped.
- The use of a mincer and a grinder for PTL4 was found to be far more effective in processing the organic fraction of the four substrates tested, including paper waste with a high lignin content.
- The grinder (macerator) produced particle size distributions (PSD) with mean PS of 1.43mm, 2.37mm, 7.05mm, and 0.97 for BPWSB, GWSB, PWSB, and TWSB for PTL3, respectively; however, such substantial particle size reduction might not be beneficial in future treatment.
- The ring size jaw opening of the mincer can alter the distribution of particle size and can affect the mid-range rather than the smaller sizes that pass through the mincer without change.
- The mincer can effectively decrease the size of feedstock particles bigger than the jaw opening, however, the output may comprise particulates that are highly irregular in shape - folded, twisted, clumped, thin, and so on.
- The mincer employed in this study for processing a wet waste fraction of PTL2 with a ring size jaw aperture RAUT 12 16# was unsuitable for handling the four-substrate biomass, especially PWSB and TWSB. This could be related to the physiochemical properties of organic materials, particularly in the case of PWSB.

There are differences in the actual PSD trends even though different particle size reduction methods have shown to be efficient in reducing the mean particle size. Many techniques profit from using diverse particle sizes at the same time. The method of biotechnological operations should be accounted for while choosing a pre-treatment method. This can promote the optimal treatment outcome for organic materials. More research is required to understand how the characteristics of various waste components throughout the size reduction process and other factors affect the particle size distribution.

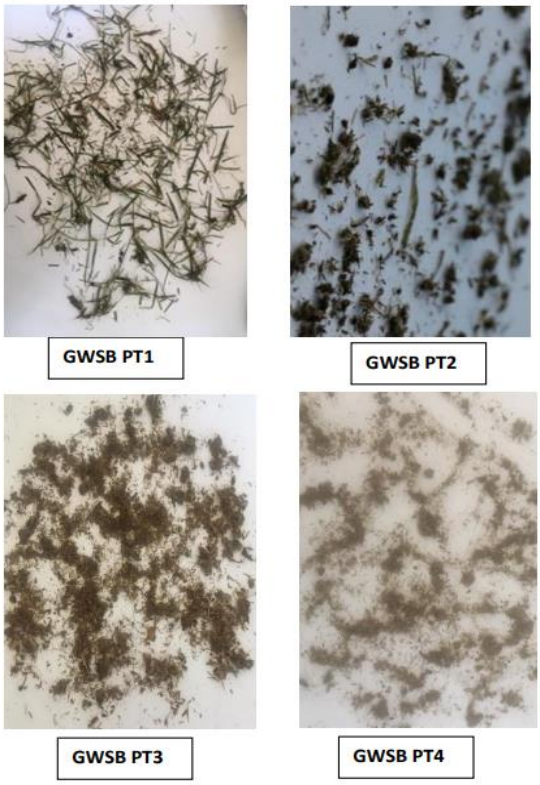
The impact of different mechanical treatments on biogas methane production across various substrates and pre-treatment levels (PTLs), results revealed a consistent trend: higher degradation rates led to increased methane output. Particularly, manually chopped BPWSB (PTL1) showed significantly higher methane production compared to other substrates, with enhancements of up to 21% over GWSB, PWSB, and TWSB. Also, processing in PTL2, PTL3, and PTL4 led to successive methane yield increases for all substrates, with the highest methane production observed in BPWSB processed in PTL4, yielding approximately 20% more methane compared to other substrates. TWSB, mainly when untreated, also exhibited notable methane production improvements compared to GWSB and PWSB. The combination of mincing and grinding (PTL4) proved most effective in enhancing methane production, outperforming other mechanical treatments. Additionally, the results stress the importance of pre-treatment in maximizing methane output, with PWSB performing best at PTL1-3, while BPWSB and TWSB excelled across all pre-treatment levels. GWSB consistently yielded the lowest methane output, highlighting the influence of substrate composition and pre-treatment methods on biogas production efficiency.

Appendices

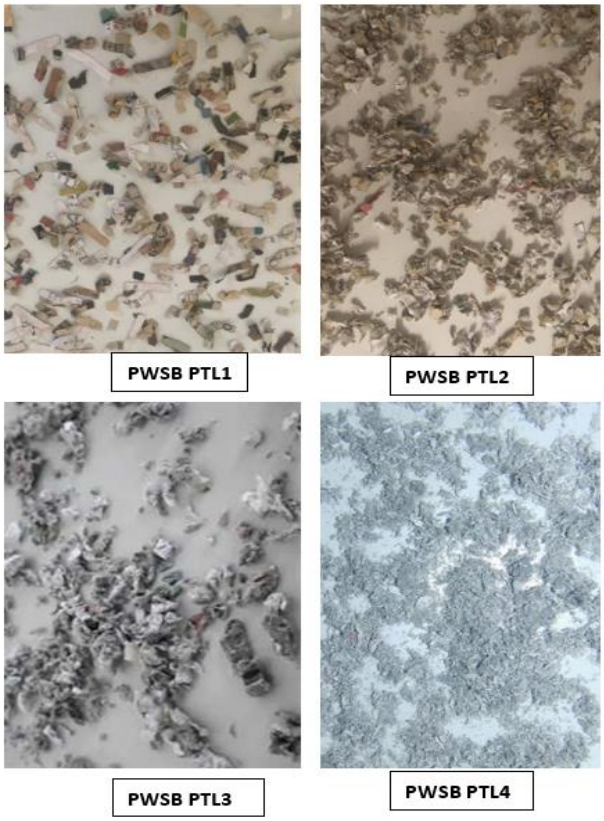
Particle Size Characterisation

Appendix A1: The photograph of each pre-treatment level of banana peel waste substrate biomass (1-4)

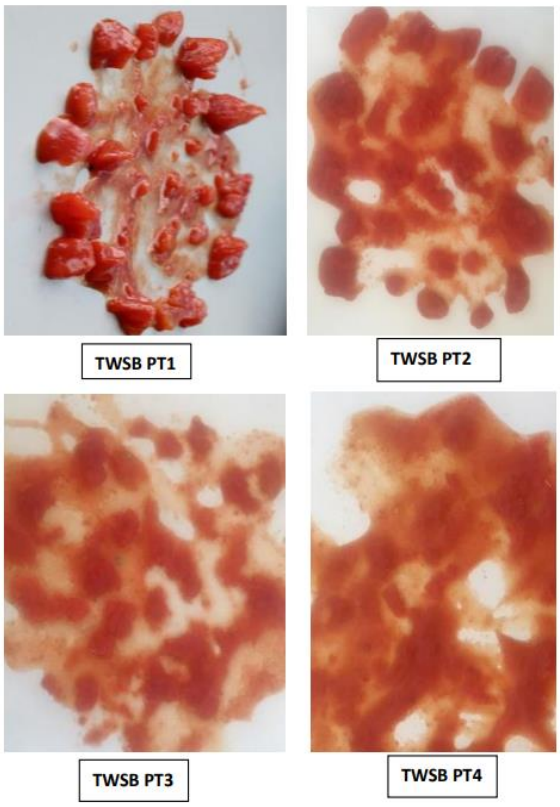
Appendix A2: The photograph of each pre-treatment level of grass waste substrate biomass (1-4)



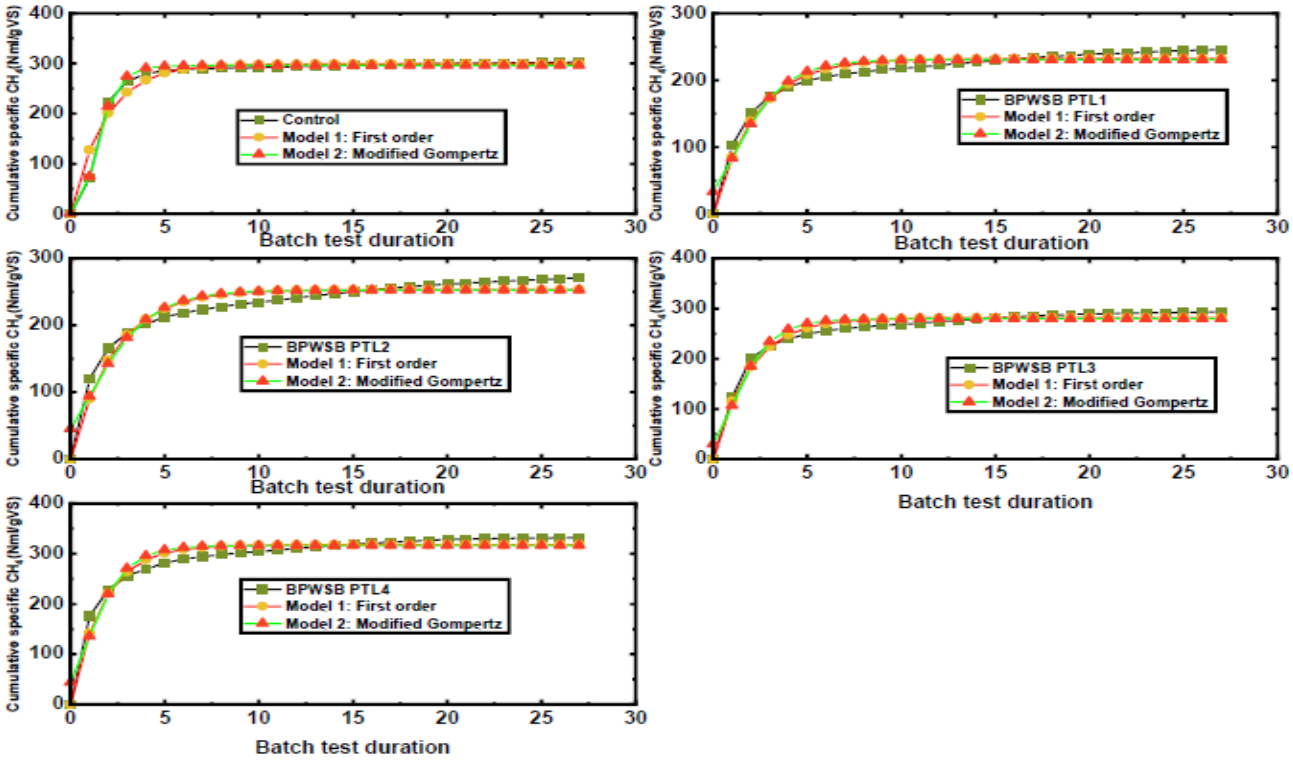
Appendix A3: The photograph of each pre-treatment level of paper waste substrate biomass (1-4)



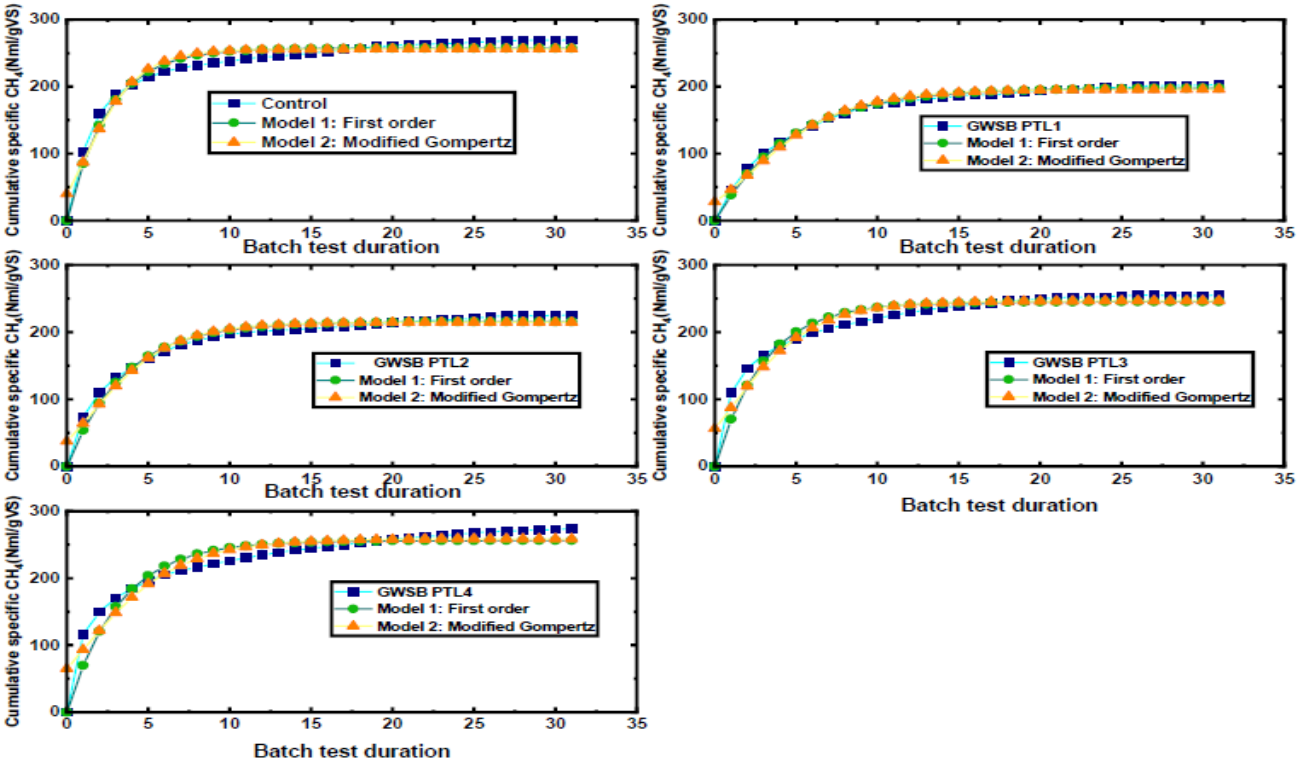
Appendix A4: The photograph of each pre-treatment level of tomato waste substrate biomass (1-4)



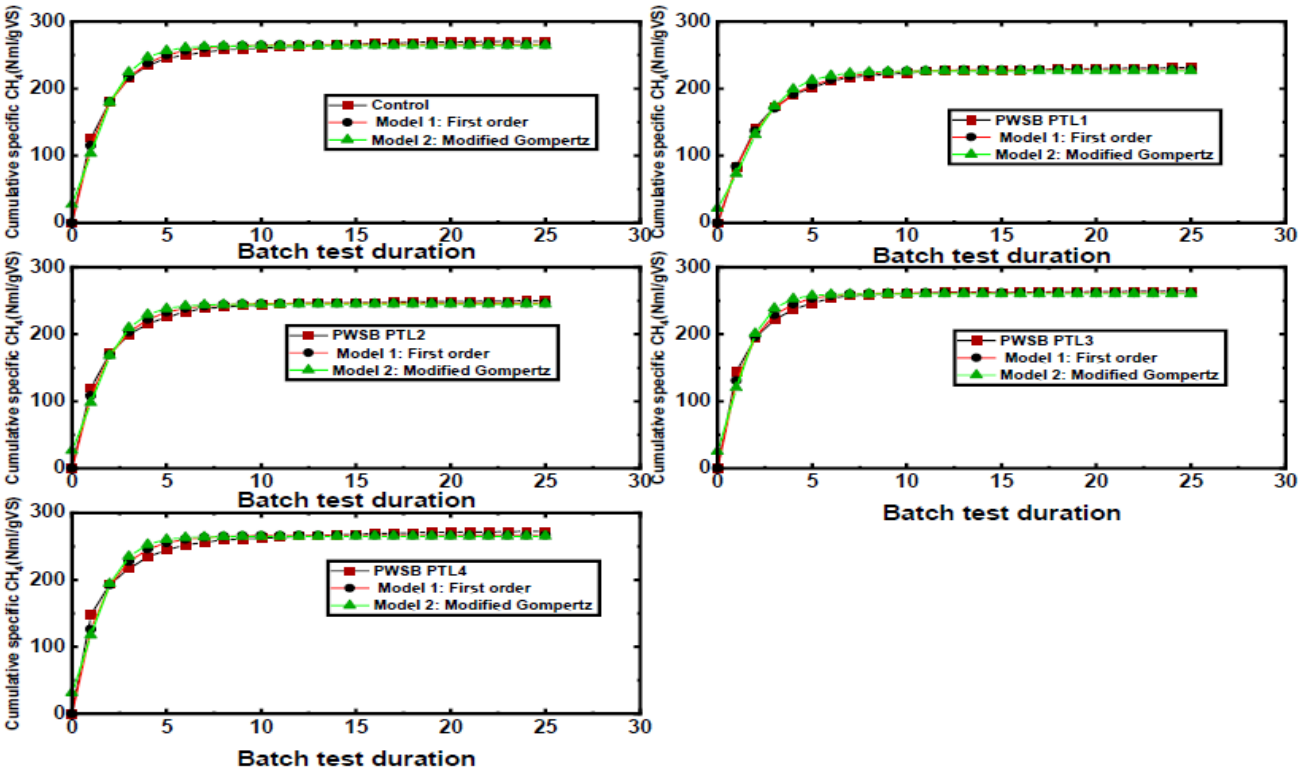
Appendix A5-1: Experimental results for BPWSB and the kinetics of specific CH4 production for first order (model 1) and modified Gompertz (model 2)



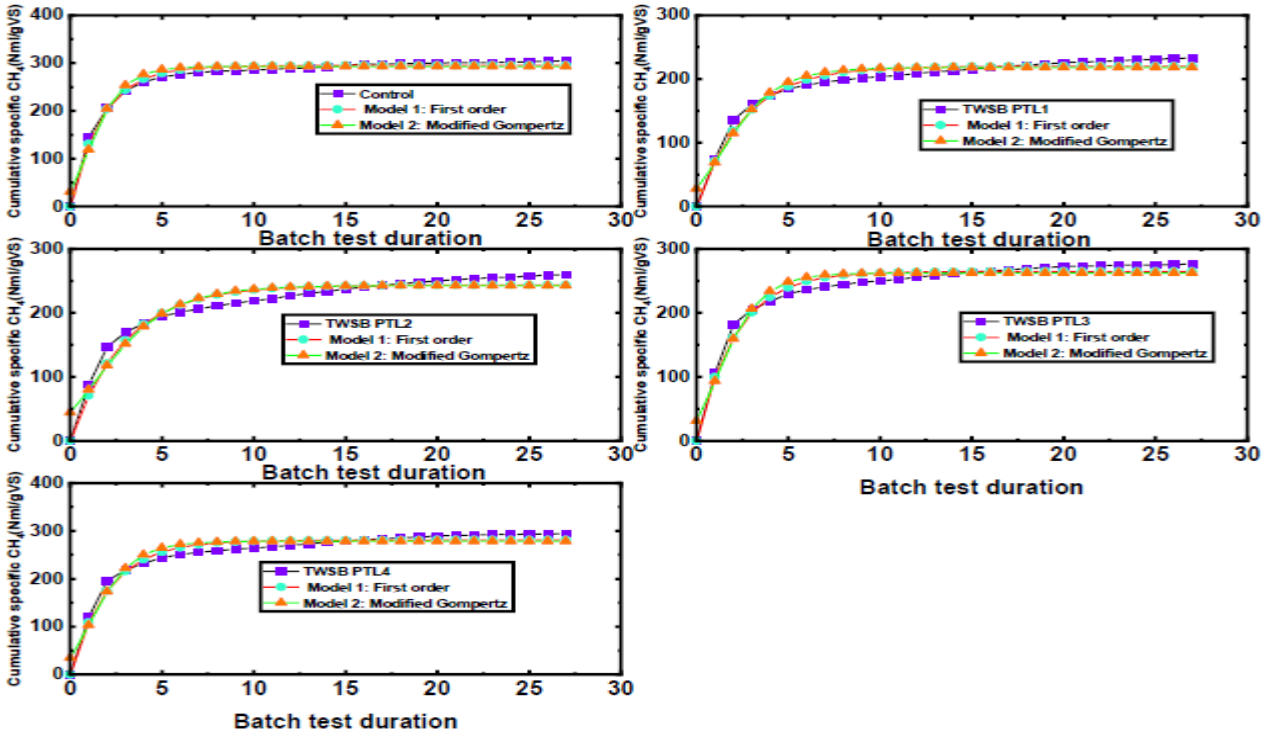
Appendix A5-2: Experimental results for GWSB and the kinetics of specific CH4 production for first order (model 1) and modified Gompertz (model 2)



Appendix A5-3: Experimental results for PWSB and the kinetics of specific CH4 production for first order (model 1) and modified Gompertz (model 2)



Appendix A5-4: Experimental results for TWSB and the kinetics of specific CH4 production for first order (model 1) and modified Gompertz (model 2)



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