Chemical Engineering Research Bulletin 24 (2024) 113-128
Available online at http://banglajol.info/index.php/CERB
DOI: https://doi.org/10.3329/cerb.v24i1.78505

Enzymatic hydrolysis for methane production from hydrolysate food waste

Nibedita Deb[1], Md. Zahangir Alam[1]*, Tawfkur Rahman[1,2], Mohammed Saedi Jami[1], and Mariatul Fadzillah Bt. Mansor[1]

[1]Bioenvironmental Engineering Research Centre (BERC), Department of Biotechnology Engineering, Faculty of Engineering, International Islamic University Malaysia, 50728 Kuala Lumpur, Malaysia

[2]Department of Electrical and Electronic Engineering, Faculty of Engineering International University of Business Agriculture and Technology, Uttara, Dhaka 1230, Bangladesh

*Correspondance: zahangir@iium.edu.my

Abstract

The study aimed to assess how different pretreatment methods affect the enzymatic breakdown of carbohydrate-rich food waste, including cellulose, glucose, starch, lignin, and hemicellulose. The goal was to use these sugars effectively to reduce methane production costs. Using kinetic modelling, the study tested various pretreatment techniques, intending to promote the economical use of waste materials in microbial fermentations, thus reducing production expenses. The findings aimed to support waste material utilization for cost reduction. Anaerobic digestion experiments were conducted using sewage sludge under stable conditions (pH 7, 30°C) for 28 days. Samples were untreated or treated with hydrolytic enzymes. After 5 days, both groups exhibited reducing sugar concentrations. The untreated group had the highest rate of production (23 day-1), while the lowest was in samples treated with 50% cellulose and 50% amylase (28 day-1). The study found that excluding nutrients substantially enhanced biogas concentrations, suggesting that using food waste without added nutrients and a 5-day hydrolysis could significantly cut production costs. However, further optimization is necessary for higher yields.

Keywords: BioMethane, Hydrolytic Enzyme, Hydrolysis, AD, OFAT, Food Waste.

1. Introduction

A significant global issue is the substantial food waste produced by restaurants, food production plants, and households, contributing to municipal solid waste (MSW). In Bangladesh, about 145 lakh tons of food are wasted annually [1], with over 37 lakh tons lost during transportation and 107 lakh tons wasted at home. OECD statistics from seven countries including the USA, Mexico, Belgium, Japan, Norway, Greece, and France reveal that organic waste constitutes 35-40% of MSW, followed by paper (28%), and smaller proportions of plastic (10%), glass (7%), and metal (5%). Food waste comprises multiple elements, with 55-67% sugar, 40-55% starch, and 2-3% cellulose [2]. Valuable components within this waste can be converted into useful products such as biogas, ethanol, and lactic acid. Studies have explored methods like enzymatic hydrolysis to recover fermentable sugars from FW and unsorted MSW, offering viable solutions to this issue [2].

Traditionally, methane has been produced from various crops like corn, wheat, sugar cane, potato and rice in different countries [4]. This process involves converting starch into sugar using commercial enzymes and fermenting the sugar into methane using activated sludge [5]. However, since these crops serve as important food sources, and utilizing abundant and cost-effective lignocellulosic waste can help reduce production costs, researchers have been exploring alternative substrates such as unsorted municipal solid waste (MSW), wheat straw, crop residues, and food waste [6]. When utilizing lignocellulosic materials, an effective enzymatic hydrolysis process typically requires a pretreatment step [7] [8]. Different pretreatment methods aim to separate or remove lignin, cellulose, and hemicelluloses, as well as reduce the crystalline structure of cellulose and increase its surface area. These improvements facilitate the penetration of hydrolytic enzymes [9]. Acid and alkaline pretreatments was demonstrated success in various studies [9]. For instance, Dawson and Boopathy (2007) employed acid (H_2SO_4) and alkaline (H_2O_2) solvents to treat sugar cane residues after harvesting and discovered that acid hydrolysis yielded higher ethanol quantities. Similarly, Deb et al.,[10] achieved high ethanol concentrations from acid-hydrolyzed cotton waste. Alternative pretreatment methods like hot water and steam pretreatment have also been explored [11].

However, when examining kitchen waste, particularly the starchy portion, previous studies [2][10] did not employ any pretreatment method prior to enzymatic hydrolysis. Kinetic models play a crucial role in understanding and predicting the performance and characteristics of a process. They offer valuable insights for controlling and forecasting these attributes. Simple methods such as assuming first-order dynamics or conducting statistical analyses can extract useful information from experimental data. The objective of kinetic modeling depends on the specific features of a chemical or biological process. In the case of pretreatment prior to enzymatic hydrolysis, the key considerations include the efficiency of the method within a short timeframe and its economic viability. The primary goal is to enhance yields in the subsequent hydrolysis step [12]. By improving the yields of enzymatic hydrolysis, the overall ethanol production can also be enhanced.

The statement emphasizes the importance of cost reduction by effectively utilizing sugars [8]. Recent studies were placed significant focus on two key areas: the utilization of lignocellulose biomass and industrial waste, and the exploration of different microbial strains during fermentation to enhance biogas production. In addition to these efforts, it was crucial to develop efficient pretreatment methods that can be practically implemented [7]. Therefore, the main objectives of the mentioned research can be summarized as follows. Firstly, the researchers aimed to evaluate the impact of enzymatic pretreatment methods, in comparison to a control group. This evaluation specifically focused on assessing the effect of these pretreatment methods on sugar production during enzymatic hydrolysis. By examining the resulting sugar production, the researchers sought to determine the effectiveness of the pretreatment methods in facilitating the breakdown of lignocellulosic materials into sugar [10]. Secondly, the research aimed to investigate the kinetics of sugar production. This involved studying the rate and timing of sugar formation during enzymatic hydrolysis. By doing so, the researchers aimed to identify the optimal timing that would enhance the enzymatic hydrolysis process before the fermentation stage. In summary, the research aimed to assess the impact of enzymatic pretreatment methods on sugar production during enzymatic hydrolysis, and to determine the most effective timing and type of pretreatment to enhance this process before fermentation. These objectives align with the broader goal of reducing costs by improving the utilization of sugars and developing efficient pretreatment methods.

2. Materials and Methods

2.1 Characterization of FW Collection

Food waste was obtained from various restaurants and supermarkets in Bangladesh and stored under controlled conditions at a temperature of 4°C to inhibit fungal growth. The waste collected comprised a diverse mixture of vegetables, fruits, cooked rice, leftovers, meat, fish, and food scraps. To initiate the process, anaerobic sludge from a local treatment plant was introduced. Since the composition of the food waste varied due to different eating habits, a reliable source of food outlets was selected. Essential parameters including, fat and oil, volatile solids, pH, total solids, protein, carbohydrates, and mineral composition were meticulously examined using well-established standardized methods.

2.2 Enzymes, inoculum, and AD medium

In the AD-based fermentation process, the fermentation medium consists of pretreated and hydrolyzed waste. The waste material undergoes pretreatment, which breaks down its complex structure and improves its digestibility. Common pretreatment techniques include physical and chemical methods. After pretreatment, the waste material undergoes hydrolysis with the help of enzymes such as amylase and cellulose [5]. These enzymes break down complex compounds into fermentable substances like sugars. The hydrolysis was performed in a plastic container, and various factors like enzyme dose, pH, and time are optimized to improve the process. The fermentation medium in biogas production includes organic waste, water for moisture balance, and essential nutrients for microbial growth and fermentation performance [10]. pH adjustment was done to maintain the optimal pH range. The fermentation medium provides the necessary environment and nutrients for microbial activity, promoting the breakdown of organic matter and the production of biogas. The composition of the fermentation medium was carefully optimized to maximize biogas yield and process efficiency [8].

2.3 Enzymatic hydrolysis

To initiate the liquefaction method of the starchy portion, an amylase enzyme was introduced into the waste at a concentration of 400 U/ml. The mixture was maintained at room temperature for 1 hour with continuous agitation at 150 rpm. The pH of the system was adjusted to 5.5 to create favorable conditions for enzymatic activity. Concurrently, the cellulosic fraction and starch-based oligosaccharides were subjected to hydrolysis using cellulase enzyme at a concentration of 326.25 U/mL. Glucose production, which serves as an indicator of reducing sugars, was monitored over time until a steady state was reached, indicating the completion of hydrolysis under the specified experimental conditions. To ensure effective enzymatic activity and substrate degradation, the entire process was conducted with continuous agitation at 150 rpm [13][14][10]. The speed at which reducing sugars were produced during enzymatic hydrolysis after pretreatment was assessed using a mathematical equation that follows first-order kinetics. This equation was employed to determine the rate of reducing sugar generation during the enzymatic hydrolysis stage after applying different pretreatment methods. The first-order dynamics refers to the mathematical model used to describe the kinetics of the reaction.

$$S = S_m(1 - e^{K\tau}) \quad (1)$$

where S represents the difference in concentration (g/L) of reducing sugar, relative to its initial concentration (S(t) S_o). Sm denotes the highest amount of reducing sugar that can be accumulated over an extremely long hydrolysis period. Meanwhile, k represents the constant rate at which reducing sugar is produced, measured in (h_1). By utilizing this equation, the researchers were able to quantify and compare the rates of glucose production under different

pretreatment conditions, providing valuable insights into the effectiveness and efficiency of the enzymatic hydrolysis process.

2.4 Fermentation

Biogas production through fermentation was a biological development that transforms organic materials into oxygen-free biogas. The main objective was to harness the energy potential of organic waste and create a sustainable source of renewable energy. Various organic materials such as agricultural residues, food waste, and animal manure are introduced into an anaerobic digester, where anaerobic bacteria and archaea decompose them through biochemical reactions [15]. These microorganisms collaborate to transform complex organic compounds into methane and carbon dioxide, resulting in the formation of biogas. Optimal fermentation conditions include factors like temperature, moisture, pH, and nutrient availability [16]. The fermentation process comprises several stages, beginning with hydrolysis, followed by acidogenesis and methanogenesis [17]. The formed biogas can be utilized for heating, electricity, or as vehicle fuel, while the byproduct, known as digestate, serves as a nutrient-rich fertilizer. Fermentation for biogas production offers an environmentally friendly solution for managing organic waste and generating renewable energy [16].

2.5 Analytical methods

Analytical methods are crucial for evaluating enzyme hydrolysis in biogas production from anaerobic digestion (AD) using food waste. They assess enzymatic efficiency, optimize biogas production, and provide valuable insights. Common methods include measuring total solids (TS) and volatile solids (VS) to determine organic content and potential biogas yield. Chemical oxygen demand (COD) indicates the digestibility and biodegradability of food waste. pH analysis helps maintain optimal conditions for enzymatic activity. Quantifying reducing sugars, like glucose, reveals hydrolysis progress and substrate availability [17][8][16]. Gas chromatography and specific tests analyses biogas composition, contaminants, and inhibitors. These methods optimize the process, ensuring efficient utilization of food waste for renewable energy generation [8].

Homogenized food waste samples were dried in an oven at 105°C until they reached a constant weight, allowing for the measurement of moisture content [18]. The pH of the fresh food waste was assessed using a Jenway 3540 bench combined conductivity/pH meter, with a sample-to-distilled water ratio of 1:10 [19]. Total nitrogen was quantified using the Kjeldahl nitrogen method (Horwitz, 2000). Freeze-dried food waste samples underwent digestion with concentrated sulfuric acid (H_2SO_4) and Kjeldahl tablets (K_2SO_4 and selenium) at a temperature of 420°C using a Gerhard infrared rapid digestion system (model TT 125M). After digestion, the mixture was diluted with water, and 40% sodium hydroxide (NaOH) was added to distill the nitrogen as ammonia (NH_3), which was subsequently captured in a 2% boric acid solution using a Velp distillation unit (model UDK219). The nitrogen content in the solution was determined through titration with 0.1 N hydrochloric acid (HCl). The crude protein (CP) content was calculated by multiplying the total nitrogen by 6.25, given that most proteins contain 16% nitrogen [20]. Crude fat (CF) content was determined via a Soxhlet extraction method using a Sanli crude fat analyser (model SZF-06B), where freeze-dried food waste samples were extracted with petroleum ether for three hours, followed by solvent evaporation at 70°C for 24 hours before weighing [21]. Total ash (TA) content was established by calculating the weight difference after dry ashing at 550°C for 18 hours using a Neytech Vulcan muffle furnace (model D-550) [21]. Total carbohydrate (TC) content was derived by subtracting the sum of protein, fat, water, and ash from 100 grams of food [21]. The ash obtained from the dry ashing process was dissolved in 6 N HCl, diluted with deionized water,

and filtered for mineral analysis (including potassium, sodium, magnesium, and calcium) using a Hitachi Z-2300 flame atomic absorption spectrophotometer [19]. Each analysis was conducted in triplicate.

Measuring the nutrient percentage of food waste involves several analytical techniques that assess the composition of organic materials discarded during food preparation and consumption. One common approach is to use proximate analysis, which evaluates moisture, protein, fat, carbohydrates, and ash content. This method typically involves drying the food waste to determine moisture content, followed by Kjeldahl digestion to quantify protein levels, and Soxhlet extraction for fat content. After determining these primary components, the carbohydrate content can be estimated by difference, and ash content provides insight into mineral composition. Recent studies have adopted advanced methods such as near-infrared spectroscopy (NIRS) and gas chromatography-mass spectrometry (GC-MS) for more precise nutrient profiling. For instance, research by Zhao et al., [22] utilized NIRS to assess the nutrient profile of food waste, highlighting its potential as a cost-effective tool for nutrient analysis. Additionally, a study by Zhao et al., [21] explored the nutritional characteristics of food waste and emphasized the importance of recycling these nutrients back into the food system to enhance sustainability. Perform proximate analysis to determine the following components as shown in Equations (3), (4), (5), (6), and (7).

$$\text{Moisture (\%)} = \left(\frac{\text{Weight of fresh sample} - \text{Weight of dry sample}}{\text{Weight of fresh sample}}\right) \times 100 \tag{3}$$

$$\text{Protein (\%)} = \left(\frac{\text{Nitrogen content} \times \text{Conversion factor}}{\text{Weight of fresh sample}}\right) \times 100 \tag{4}$$

$$\text{Fat Content (\%)} = \left(\frac{\text{Weight of fat extracted}}{\text{Weight of fresh sample}}\right) \times 100 \tag{5}$$

$$\text{Ash Content (\%)} = \left(\frac{\text{Weight of ash}}{\text{Weight of fresh sample}}\right) \times 100 \tag{6}$$

$$\text{Carbohydrates (\%)} = 100 - (\text{Moisture \%} + \text{Protein \%} + \text{Fat \%} + \text{Ash \%}) \tag{7}$$

For accurate measurements, food waste samples should be collected from diverse sources, including households, restaurants, and food processing facilities, to ensure a representative analysis of nutrient content. Ultimately, these measurements not only aid in understanding the nutritional potential of food waste but also inform strategies for reducing waste and enhancing resource recovery in food systems.

3. Results and discussion

3.1 Composition of food waste material

Food waste is a complex phenomenon that warrants comprehensive characterization. Understanding the intricacies of food waste can shed light on its causes, impacts, and potential solutions [22]. This text aims to present a revised analysis of food waste, delving into its various dimensions. Food waste material is composed of various organic components that are discarded from the food industry, households, and commercial establishments [23]. The specific composition of food waste can vary depending on factors such as geographic location, cultural practices, and individual preferences [24]. Table 1 presents the composition of food waste, with

an average moisture content of approximately 79.53% (w/w), leaving 35% (w/w) as total dry matter. The carbohydrate portion constituted around 25.4% of the total dry matter, highlighting the potential of FW as a valuable source for biogas manufacture.

Table 1: Analysis of Food Waste

Parameters	Units	Concentration
Total Solids (TS)	(% w/v)	15.6
Volatile Solids (VS)	(%w/v)	13.62
Moisture Contain	(%)	79.53
Reducing Sugars	mg/mL	16.5
pH	-	5.05
Proteins	(%w/v)	9.7
Chemical Oxygen Demand (COD)	g/L	89.2
Carbohydrates	(%w/v)	25.4
Ash	(%)	1.03
Total nitrogen	(%)	3

The analysis of the food waste substrate reveals several key characteristics that are important for understanding its potential in biogas production [24]. The total solids (TS) content is measured at 15.6% (w/v), indicating a relatively high concentration of solid material in the waste [25]. Of this, the volatile solids (VS), which represent the organic portion that can be decomposed by microorganisms, account for 13.62% (w/v). The moisture content of the substrate is significant at 79.53%, suggesting that the material is quite wet, which is typical for food waste and beneficial for anaerobic digestion processes [26]. The concentration of reducing sugars, a critical factor for fermentation efficiency, is 16.5 mg/mL, while the pH of the substrate is slightly acidic at 5.05. The protein content, an essential nutrient for microbial growth, is measured at 9.7% (w/v), further supporting its potential use in biogas production [27]. In addition to organic content, other parameters also play a crucial role in assessing the substrate's quality for biogas generation. The chemical oxygen demand (COD), which reflects the amount of oxygen required to oxidize organic compounds, is notably high at 89.2 g/L, indicating a significant organic load available for microbial breakdown [28]. Carbohydrates, a primary source of energy for microbes, comprise 25.4% (w/v) of the substrate, while the ash content, representing inorganic material, is relatively low at 1.03%. The total nitrogen content, crucial for balancing the carbon-to-nitrogen ratio in anaerobic digestion, is 3%, suggesting the substrate has sufficient nitrogen for microbial activity [29]. These parameters collectively demonstrate that the food waste substrate is well-suited for biogas production, with its high organic content and favorable chemical composition providing an ideal environment for methane generation [30][31] [32[33]. As per Lin et al., [30], the majority of biodegradable waste is commonly either sent to landfills or recycled and utilized in first-generation processes like organic manure, biofuels, or livestock feed. However, these practices have adverse social and environmental implications, leading to the release of pollutants into water air, and soil. It has been estimated that each metric ton of biodegradable waste disposed of in landfills emits 4.2 metric tons of carbon dioxide (CO2), contributing to 3% of global greenhouse gas (GHG) emissions [5]. To address these issues, the waste was mixed with water in a 1:1 (v/v) ratio and crushed into smaller particles to minimize its impact. Subsequently, it underwent digestion at a temperature of 55°C for a duration of 12 hours, resulting in a hydrolysate with a high sugar content of 9 g/L [30][31].

3.2 Effect of enzymatic hydrolysis methods on sugar production

The methods of enzymatic hydrolysis have a substantial influence on the production of sugars when complex carbohydrates are converted into simpler forms. The choice of enzymatic hydrolysis method can influence the efficiency and yield of sugar production. Different factors such as the type of enzyme used, enzyme dosage, reaction conditions (pH, temperature, duration), and substrate characteristics can affect the effectiveness of enzymatic hydrolysis and subsequent sugar production. Optimizing enzymatic hydrolysis methods is crucial to maximize sugar yields [31]. The selection of appropriate enzymes based on the substrate composition is important. Different enzymes have varying specificities for different types of carbohydrates, such as amylases for starch hydrolysis or cellulases for cellulose hydrolysis. Using a combination of enzymes with complementary activities can enhance the hydrolysis efficiency.

The variations in reducing sugar concentration were examined during the enzymatic hydrolysis of pretreated samples at different pH levels and over a specific duration, as depicted in Fig. 1. Over time, the concentration of reducing sugar gradually increased and eventually stabilized within three hours for all enzymatic pretreatment methods [32]. The highest concentration of reducing sugar, 72.3 g/L, was obtained at pH 5.5 after 3 hours of hydrolysis for the unpretreated samples. Other parameters such as enzyme dose (80 U/mL), agitation (150 rpm), and room temperature (30°C) were kept constant. Figure 1 clearly indicates a decline in reducing sugar concentration between 4 to 5 hours and at pH levels 6 to 7. The decrease in reducing sugar concentration at pH 6, pH 6.5, and pH 7 to values of approximately 58 g/L, 44 g/L, and 57 g/L, respectively, can be attributed to the effect of pH on enzymatic activity and substrate availability. Enzymes involved in the hydrolysis of pretreated samples are highly sensitive to changes in pH. Different pH levels can affect the conformation and stability of enzymes, influencing their catalytic efficiency. In this case, it appears that the enzymes responsible for the hydrolysis of the pretreated samples were less efficient at pH 6, pH 6.5, and pH 7 compared to pH 5.5. Moreover, the pH of the reaction environment also impacts the structure of the substrate molecules. Changes in pH can alter the accessibility and susceptibility of the substrate to enzymatic cleavage. It is possible that at pH 6, pH 6.5, and pH 7, the substrate molecules were less favorable for enzymatic hydrolysis, resulting in reduced sugar release compared to pH 5.5. Additionally, it is important to consider that pH affects the overall chemical equilibrium of the reaction. At certain pH levels, the equilibrium between hydrolysis and reverse reactions can shift, influencing the overall yield of reducing sugars. Therefore, the observed decrease in reducing sugar concentration at pH 6, pH 6.5, and pH 7 can be attributed to the combined effects of pH on enzymatic activity, substrate structure, and reaction equilibrium.

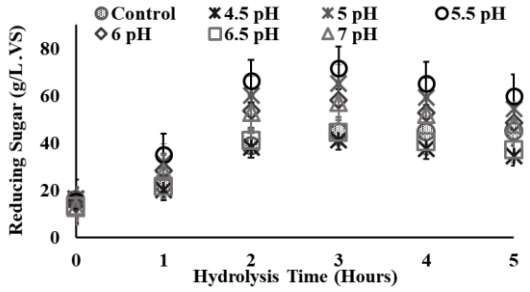

Fig. 1: Reducing sugar production from hydrolyzed wastes subjected to different pH and hydrolysis time. Other factors were fixed such as enzyme dose 80U/mL, agitation 150 rpm and room temperature 30°C.

In Fig. 1, the concentration of reducing sugars produced from hydrolyzed wastes is evaluated under varying pH levels and hydrolysis time. The results indicate that as the pH shifts from acidic to neutral and potentially alkaline conditions, the production of reducing sugars exhibits notable changes. Optimal pH conditions typically enhance enzyme activity, facilitating the hydrolysis of complex carbohydrates into simpler sugars. At lower pH levels, enzyme activity may be hindered, resulting in lower reducing sugar concentrations. Conversely, an optimal pH range (around 4.5 to 7) tends to maximize reducing sugar production. At higher pH levels, while some enzymes remain effective, others may become denatured, leading to a decline in sugar production. This interplay illustrates the significance of pH in enzymatic hydrolysis processes. Enzyme dosage is another critical factor. Insufficient enzyme dosage may result in incomplete hydrolysis and lower sugar yields, while excessive dosage can be costly and may lead to non-specific reactions. Finding the optimal enzyme dosage through experimentation is essential for achieving high sugar production. Reaction conditions, including pH, temperature, and duration, also impact enzymatic hydrolysis [8].

In Fig. 2 shows the production of reducing sugars from hydrolyzed wastes under varying temperatures showed a distinct trend influenced by incubation time and temperature. When temperatures were held constant at 20°C, 25°C, 30°C, 35°C, 40°C, and 45°C, while other factors like pH (5.5), enzyme dose (80U/mL), and agitation speed (150 rpm) were fixed, there was a clear enhancement in sugar production with higher temperatures and longer incubation times. After 1 hours of incubation, reducing sugar production ranged from 16.1 g/L in the control to 22.54 g/L at 30°C, indicating a rapid increase up to moderate temperatures. By 3 hours, the trend intensified, with 82.62 g/L of reducing sugars recorded at 30°C, making it the most efficient temperature for sugar production, while 45°C showed a decrease, likely due to enzyme denaturation or substrate instability at higher temperatures [4][22][33].

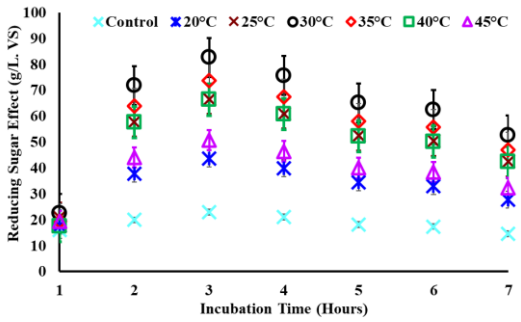

Fig. 2.: Reducing sugar production from hydrolyzed wastes subjected to different temperature. Other factors were fixed such as pH 5.5, enzyme dose 80U/mL and agitation 150 rpm.

As the incubation period extended beyond 3 hours, the reducing sugar levels started to decline across all temperatures. By 7 hours, reducing sugar production fell, with 52.67 g/L observed at 30°C, while higher temperatures like 40°C and 45°C produced even less at 46.97 g/L and 32.34 g/L, respectively. This suggests that prolonged exposure to higher temperatures, though initially beneficial, can lead to diminishing returns in sugar production, potentially due to the degradation of the enzyme or substrate over time. The control, which lacked temperature treatment, exhibited consistently lower sugar production across all incubation periods, highlighting the significant impact of optimal temperature conditions on enzymatic hydrolysis efficiency [23][24].

In Fig. 3 demonstrations the production of reducing sugars from hydrolyzed wastes subjected to varying agitation speeds demonstrated a clear impact on sugar yield, with higher agitation generally promoting increased hydrolysis efficiency. Over a 7-hours incubation period, and under fixed conditions of pH 5.5, enzyme dose of 80U/mL, and temperature of 30°C, agitation rates ranging from 50 rpm to 300 rpm were examined. At 1 hour, sugar production increased as agitation moved from 50 rpm (17.71 g/L) to 150 rpm (22.54 g/L), suggesting that moderate agitation facilitates better enzyme-substrate interaction. However, higher agitation speeds like 200 rpm and 300 rpm did not significantly improve sugar production, with reducing sugar levels at 19.48 g/L, likely due to shear forces disrupting enzyme stability or substrate availability [5][8].

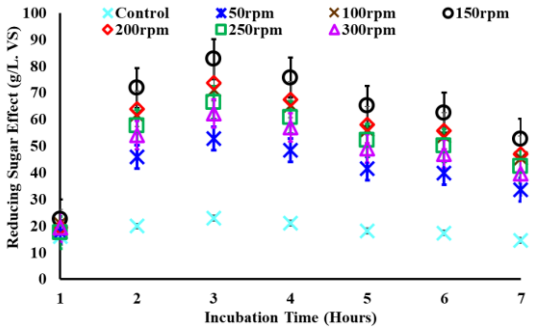

Fig.3: Reducing sugar production from hydrolyzed wastes subjected to different agitation. Other factors were fixed such as pH 5.5, enzyme dose 80U/mL, and room temperature 30°C

By 3 hours, the trend became more pronounced, with the highest sugar production (82.62 g/L) achieved at 150 rpm, while lower agitation rates, such as 50 rpm (52.79 g/L) and 100 rpm (66.56 g/L), showed progressively less effectiveness. Beyond this point, increasing agitation to 200 rpm and higher resulted in diminishing returns, with 250 rpm yielding 66.33 g/L and 300 rpm at 62.19 g/L. This suggests that optimal agitation enhances the hydrolysis process by maximizing enzyme activity and ensuring proper substrate mixing, but excessive agitation can lead to enzyme deactivation or mechanical stress on the substrate. As incubation extended to day 7, sugar production continued to drop across all agitation levels, indicating that the hydrolysis process was reaching completion [25][33].

Optimizing enzyme activity in hydrolysis involves several key strategies. First, adjusting pH and temperature to align with the enzyme's optimal range enhances its efficiency, as enzymes have specific conditions under which they function best. Fine-tuning the enzyme dose is also essential, as too much or too little can lead to inefficiencies [31]. Pre-treating substrates, such as through physical, chemical, or thermal methods, can improve enzyme access and boost conversion rates. Using enzyme cocktails that combine different enzymes can enhance breakdown by targeting multiple substrate components simultaneously [32]. Additionally, immobilizing enzymes on solid supports allows for reuse and greater stability, while proper agitation ensures uniform mixing and prevents enzyme deactivation. Enhancing enzyme stability through additives or genetic modifications, and using fed-batch systems for continuous enzyme supply, are further effective strategies for optimizing hydrolysis efficiency. Enzymes have specific pH and temperature optima at which they exhibit maximum activity [8]. Maintaining these optimal conditions can enhance enzyme performance and increase sugar yields as depicted in Fig. 4. Initially, increasing the enzyme dose from 40 U/mL to 80 U/mL resulted in higher reducing sugar concentrations of 44 g/L, 65 g/L, and 75.8 g/L at 1 hour, 2

hours, and 3 hours, respectively. The highest concentration of reducing sugar, 75.8 g/L, was obtained with an enzyme dose of 80 U/mL, while keeping other parameters constant, including a hydrolysis time of 3 hours, pH 5.5, agitation at 150 rpm, and a room temperature of 30°C. Subsequently, when the enzyme dose was further increased from 100 U/mL to 140 U/mL, the reducing sugar concentrations decreased. At a hydrolysis time of 3 hours, the reducing sugar concentrations were approximately 58.16 g/L, 44.37 g/L, and 41.26 g/L. As the hydrolysis time was extended to 4 hours and 5 hours, the reducing sugar concentrations further declined to 52.85 g/L, 40.32 g/L, and 37.65 g/L at 4 hours, and 48.60 g/L, 37.08 g/L, and 33.25 g/L at 5 hours, respectively.

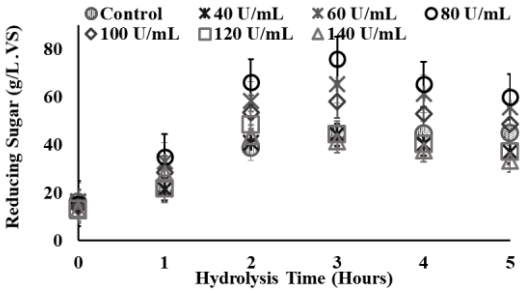

Fig. 4: Reducing sugar production from hydrolyzed wastes subjected to different enzyme dose. Other factors were fixed such as pH 5.5, agitation 150 rpm and room temperature 30°C.

In Fig. 4, the impact of varying enzyme doses on the production of reducing sugars from hydrolyzed wastes is analyzed while keeping other factors constant at pH 5.5, agitation speed of 150 rpm, and a temperature of 30°C. The concentration of reducing sugars typically increases with higher enzyme doses, as more enzymes are available to catalyze the breakdown of complex substrates into simpler sugars. However, there is a threshold beyond which additional enzyme doses may not significantly enhance sugar production due to substrate saturation or enzyme inactivation. This means that while increasing enzyme concentration generally leads to higher reducing sugar yields, there can be diminishing returns once the optimal enzyme concentration is reached. Ultimately, the enzyme dose plays a crucial role in optimizing the efficiency of the hydrolysis process, with careful consideration needed to balance enzyme availability and substrate concentration. Increasing the enzyme dose from 40 U/mL to 80 U/mL initially led to higher reducing sugar concentrations. However, when the enzyme dose was increased beyond 80 U/mL, the reducing sugar concentrations started to decrease. Moreover, as the duration of hydrolysis extended from 3 hours to 4 hours and 5 hours, there was a gradual decrease in the concentrations of reducing sugars. These findings suggest a complex relationship between enzyme dosage, hydrolysis time, and the production of reducing sugars during the enzymatic hydrolysis process. Additionally, the duration of hydrolysis influences the extent of sugar production. According to Wu et al., [33] and Zhao et al., [23], extending reaction times can increase sugar yields, but excessively long durations may lead to diminishing returns. In their study, they found that after a period of 7 days of hydrolytic acidification, there was a significant reduction of 48.1% in volatile solids. From this process, a yield of 6.8 g/L of volatile fatty acids, 82 g/L of reducing sugars (RS), and 4.7 g/L of acetic acid was obtained. Furthermore, research focusing on enzyme digestion has indicated that growth substrates contribute to approximately 40% of the overall manufacturing costs [22][23]. To address this issue and reduce costs, it is crucial to explore alternative low-cost substrates for industrial applications.

Table 2 presents the determined parameters of the first-order kinetics model for the enzymatic hydrolysis process following each pretreatment method. According to the table, it was observed that the control group, treated with a combination of cellulose and amylase (no pretreatment), showed higher glucose concentrations compared to the enzymatically pretreated samples ($p < 0.05$).

Table 2: The parameters of the kinetics model were determined for the enzymatic hydrolysis process.

Enzymatic Hydrolysis	Sm (g/L)	R2	K(h-1)	τ (h)
Control	44.61	0.989	0.544	2.05
Combine enzyme (50%+50%)	71.80	0.968	0.228	3.91

Nevertheless, there was no significant distinction in glucose concentrations between the two enzyme doses of 80U/mL. Both doses demonstrated comparable glucose concentrations ($p > 0.05$). The findings of an experiment that examined how glucose concentration changed over time during enzymatic hydrolysis samples. The data was presented using Fig. 1 and Fig. 2. As time progressed, the reducing sugar concentration slowly increased and eventually reached a steady level within a 3 h time when enzymatic pretreatment methods were used. Out of all the samples, the unpretreated ones demonstrated the highest reducing sugar concentration of 71.80 g/L after 3 hours [24]. However, when comparing the various samples, the observed reducing sugar concentrations were statistically similar, as indicated by a p-value greater than 0.05 (<0.05). These values fell within the margin of error provided by Tukey's confidence interval, although it is important to note that they were on the borderline, implying that they could be considered practically distinct. As a result, the researchers concluded that enzymatic treatment is an effective approach for managing mixed food wastes, based on a prior study by Dawson and Boopathy, [9]. In the mentioned study, enzymatic pretreatment was applied to post-harvest sugarcane residue before the fermentation process.

In order to analyze the data presented in Fig. 1, Fig. 2, Fig. 3 and Fig. 4 using Equation (1), a transformation was applied by subtracting the initial concentration from the obtained reducing sugar values at each specific time point, as shown in Table 2. To enhance understanding, it is important to explicitly discuss the kinetic models applied, along with their corresponding equations. Common kinetic models used in enzymatic hydrolysis Equation (1), which describes the rate of enzyme-catalyzed reactions. Additionally, other models, such as the first-order kinetics or zero-order kinetics, should be discussed depending on the data characteristics observed. Providing these equations and explaining their relevance to the observed kinetic behavior will help clarify the analysis and allow for a more robust interpretation of the results shown in Fig. 5. Notably, all the curves in the transformed form intersected at the origin. The results of the kinetic analysis for the enzymatic pretreatment method are provided in Table 2. As expected, the findings indicated that the rate of reducing sugar production was higher when an enzyme dose of 80u/mL was utilized for both enzymes. However, the final concentrations of reducing sugar for both enzyme pretreatments were similar, as depicted in Fig. 1, Fig. 2, Fig. 3 and Fig. 4. The rate constants can be ranked in descending order as follows: $k_{Control} > k_{ED}$% for the cases of no pretreatment (Control) and an enzyme dose of 80U/mL, respectively. When examining the time constant (s), which represents the duration needed for the reducing sugar level to reach 71.81% of the final steady level during the hydrolysis process, the control method displayed the lowest time constant value of 2.05 hours.

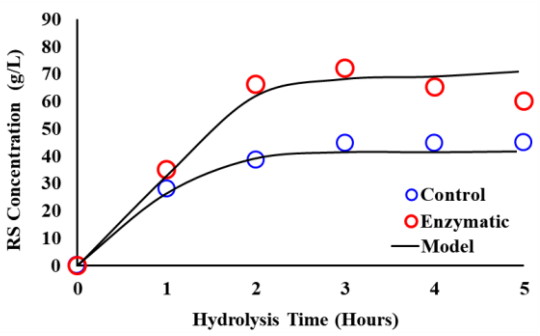

Fig. 5: Transformed results for reducing sugar production after enzymatic treatment method

In contrast, the enzymatic pretreatment method had the highest value of 3.91 hours, consistent with the rate constants [25][26]. While pH is a critical factor influencing enzymatic hydrolysis and sugar production, it should not be the only variable examined. Other parameters, such as temperature, enzyme concentration, substrate type, agitation speed, and reaction time, also play significant roles in optimizing hydrolysis efficiency and maximizing reducing sugar yields [4-5]. For instance, temperature can affect enzyme activity and stability, while enzyme concentration directly influences the rate of substrate breakdown [4]. Additionally, the nature of the substrate can determine how effectively enzymes can access and hydrolyze complex carbohydrates. Therefore, a comprehensive approach that considers multiple variables is essential for accurately assessing the effects of enzymatic hydrolysis on sugar production and for optimizing the overall process [5].

3.3 Effect of Fermentation on biogas production

Following the completion of the enzymatic hydrolysis process, the pretreated and hydrolyzed samples were subjected to batch biogas production. This involved utilizing a biogas inoculum of 10%, maintaining a pH level of 7, conducting anaerobic digestion for a duration of 30 days, and keeping the temperature at room temperature, specifically 30°C (with a variation of ±2°C). The objective was to evaluate the fermentability of the hydrolysates and determine whether additional chemical nutrients were necessary in the fermentation medium. The experimental design details and the outcomes of the fermentation experiments are provided in Table 3. The initial reducing sugar concentration, final biogas concentration, and yield values are provided in Table 3 as well. A statistical analysis of the data revealed that regardless of the pretreatment method used ($p > 0.05$), there were no significant differences in biogas concentrations and yields between the samples with added nutrients and those without.

Table 3: Fermentation effects of food wastes exposed to enzymatic hydrolysis.

Pretreatment	Reducing Sugar before fermentation (mg/L)	Biogas (mg/L)	Bio methane (%)
Control	44.61	288	52 (149.76mg/L)
Enzyme hydrolysis	71.80	600	72 (432mg/L)

The results indicate a significant difference in reducing sugar concentrations before fermentation between the control and enzyme hydrolysis pretreatment methods. The control sample exhibited a reducing sugar concentration of 44.61 mg/L, whereas the enzyme hydrolysis treatment increased this concentration to 71.80 mg/L. This enhancement in sugar

availability is crucial, as higher reducing sugar levels can facilitate more efficient fermentation processes, leading to greater biogas production. The increase in reducing sugars indicates that the enzymatic hydrolysis effectively breaks down complex carbohydrates into simpler sugars, making them more readily accessible for microbial fermentation. In terms of biogas production, the enzyme hydrolysis pretreatment resulted in a substantial increase in biogas yield compared to the control group. The control produced 288 mg/L of biogas, while the enzyme hydrolysis treatment yielded 600 mg/L. This enhancement correlates with the increase in reducing sugar concentration, as the availability of fermentable sugars directly impacts the biogas output. Furthermore, the bio-methane percentage increased from 52% in the control to 72% in the enzyme hydrolysis treatment, demonstrating that not only did the total biogas volume increase, but the quality of the biogas improved as well, as reflected in the higher concentration of methane (432 mg/L). These findings underscore the importance of pretreatment methods, particularly enzymatic hydrolysis, in optimizing both biogas production and bio-methane quality from organic substrates. In a separate study by Yang et al., [27], similar fermentation experiments with low biogas concentration (110 mL/g) were conducted to assess the fermentability of hydrolysates, but no optimization efforts were made. The biogas volume obtained from the anaerobic digestion (AD) of hydrolyzed food waste showed a significant increase compared to previous studies. Pavi et al., [28] reported a cumulated biogas volume of 493.8 mL/g VS at pH 7 from fruit and vegetable waste, while Kiran et al., [29] observed a cumulated biogas volume of 468.2 mL/g RS at pH 7 from food waste. In contrast, Yang et al., [27] found a much lower cumulative biogas volume of only 171.0 mL/g TS at pH 7 from food waste collected from a canteen, which included rice, meats, vegetables, bones, and other components.

4. Conclusion

The results of this study suggest that an enzymatic hydrolysis step followed by acid and base pretreatment methods may not be essential for achieving high production of reducing sugars from the examined food waste. The hydrolysis process can be effectively completed within a short period of 3 hours, pH 5.5, agitation 150 rpm and room temperature 30°C, resulting in a significant increase in reducing sugar concentration from 44.61 mg/L in the control sample to 71.80 mg/L with enzyme hydrolysis. However, it was found that the addition of fermentation nutrients is crucial for anaerobic sludge to generate methane. The original food waste contained insufficient nutrients, leading to a biogas yield of 288 mg/L and a bio-methane concentration of 52%. In contrast, with the inclusion of additional nutrients, the biogas production increased to 600 mg/L, and the bio-methane concentration rose to 72%, emphasizing the importance of nutrient supplementation. Therefore, this study concludes that by utilizing food waste as a substrate and eliminating the conventional practice of relying solely on fermentation anaerobic sludge, the costs associated with methane production could be significantly reduced. The enhanced biogas yield of 600 mg/L and higher bio-methane quality demonstrate the potential for optimizing biogas production processes, making food waste a viable and cost-effective renewable energy source. By focusing on the optimization of nutrient conditions, this approach not only improves methane yield but also contributes to more sustainable waste management practices.

Acknowledgement

The authors are grateful to the Department of Biotechnology Engineering for providing the lab facilities and the Biotechnology Engineering Research Center (BERC), IIUM, for providing

the funds. We present our gratitude to the project fund ID: FRGS19-106-0715 has been supported by the Ministry of Higher Education, Malaysia.

5. References

[1] Shamsuzzaman, M. M., Mozumder, M. M. H., Mitu, S. J., Ahamad, A. F., & Bhyuian, M. S. (2020). The economic contribution of fish and fish trade in Bangladesh. Aquaculture and Fisheries, 5(4), 174-181.

[2] Wang, Q., Ma, H., Xu, W., Gong, L., Zhang, W., & Zou, D. (2008). Ethanol production from kitchen garbage using response surface methodology. Biochemical Engineering Journal, 39(3), 604-610.

[3] Varga, E., Thomsen, A. B., & Feng, L. (2005, May). Ethanol production from corn, corn stover and corncob from the Jilin province of China. In Technologies for sustainable energy development in the long term. Proceedings, Riso International Energy Conference (pp. 369-378). Denmark: Riso National Laboratory.

[4] Deb, N., Alam, M. Z., Al-khatib, M. F. R., & Elgharbawy, A. (2019). Development of Acid-Base-Enzyme Pretreatment and Hydrolysis of Palm Oil Mill Effluent for Bioethanol Production. Liquid Biofuel Production, 197-217.

[5] Deb, N., Alam, M. Z., Rahman, T., Al-Khatib, M. A. F. R., Jami, M. S., & Mansor, M. F. B. (2023). Acid–Base Pretreatment and Enzymatic Hydrolysis of Palm Oil Mill Effluent in a Single Reactor System for Production of Fermentable Sugars. International Journal of Polymer Science, 2023.

[6] Jensen, J. W., Felby, C., Jørgensen, H., Rønsch, G. Ø., & Nørholm, N. D. (2010). Enzymatic processing of municipal solid waste. Waste Management, 30(12), 2497-2503.

[7] Sun, F., & Chen, H. (2007). Evaluation of enzymatic hydrolysis of wheat straw pretreated by atmospheric glycerol autocatalysis. Journal of Chemical Technology & Biotechnology: International Research in Process, Environmental & Clean Technology, 82(11), 1039-1044

[8] Deb, N., Rahman, T., Alam, M. Z., Miah, M. S., & Kamal, R. (2024). A Single-Reactor System for Simultaneous Pretreatment and Fermentation of POME for Bioethanol Production. International Journal of Polymer Science, 2024(1), 5264918.

[9] Dawson, L., & Boopathy, R. (2007). Use of post-harvest sugarcane residue for ethanol production. Bioresource technology, 98(9), 1695-1699.

[10] Deb, N., Alam, M. Z., Rahman, T., Jami, M. S., Mansor, M. F. B., & Tajuddin, H. B. A. (2023). Anaerobic Digestion for Biomethane Production from Food Waste Pretreated by Enzymatic Hydrolysis. Journal of Biotechnology Research, 9(1), 6-20.

[11] Deb, N., Rahman, T., Alam, M. Z., Miah, M. S., & Kamal, R. (2024). A Single-Reactor System for Simultaneous Pretreatment and Fermentation of POME for Bioethanol Production. International Journal of Polymer Science, 2024(1), 5264918.

[12] Alam, M. Z., & Dev, N. (2020). Solid State Bioreactor for Food Waste Composting Process: Process Optimization. Journal of Energy and Environment, 12(2), 2020.

[13] Yan, S., Li, J., Chen, X., Wu, J., Wang, P., Ye, J., & Yao, J. (2011). Enzymatical hydrolysis of food waste and ethanol production from the hydrolysate. Renewable Energy, 36(4), 1259-1265.

[14] Kim, J. H., Lee, J. C., & Pak, D. (2011). Feasibility of producing ethanol from food waste. Waste management, 31(9-10), 2121-2125.

[15] Rahman, T., Alam, M. Z., Moniruzzaman, M., Miah, M. S., Horaira, M. A., & Kamal, R.(2024). Navigating the Contemporary Landscape of Food Waste Management in Developing Countries: A Comprehensive Overview and Prospective Analysis. Heliyon, 10(12), e33218.

[16] Buenrostro-Figueroa, J. J., Nevárez-Moorillón, G. V., Chávez-González, M. L., Sepúlveda, L., Ascacio-Valdés, J. A., Aguilar, C. N., ... & Arely Prado-Barragán, L. (2023). Improved Extraction of High Value-Added Polyphenols from Pomegranate Peel by Solid-State Fermentation. Fermentation, 9(6), 530.

[17] Koupaie, E. H., Lakeh, A. B., Azizi, A., Hafez, H., & Elbeshbishy, E. (2022). Integrated two-phase acidogenic-methanogenic treatment of municipal sludge with thermal hydrolysis. Waste Management, 144, 173-181.

[18] Allen, S. E., Grimshaw, H. M., Parkinson, J. A., & Quarmby, C. (1974). Chemical analysis of ecological materials (pp. xii+-565).

[19] Cunniff, P., & Washington, D. (1997). Official methods of analysis of AOAC International. J. AOAC Int, 80(6), 127A.

[20] Nollet, L. M. (2004). Handbook of Food Analysis: Volume 1: Physical Characterization and Nutrient Analysis. CRC press.

[21] Nielsen, S. S., Eds. 2003. Food analysis. 3rd ed. New York: Kluwer Academic/Plenum Publishers.

[22] Zhao, Z., Qi, S., Wang, R., Li, H., Song, G., Li, H., & Yin, Q. (2024). Life cycle assessment of food waste energy and resource conversion scheme via the integrated process of anaerobic digestion and hydrothermal carbonization. International Journal of Hydrogen Energy, 52, 122-132.

[23] Zhao, J., Jing, Y., Zhang, J., Sun, Y., Wang, Y., Wang, H., & Bi, X. (2019). Aged refuse enhances anaerobic fermentation of food waste to produce short-chain fatty acids. Bioresource technology, 289, 121547

[24] Aswathy, U. S., Sukumaran, R. K., Devi, G. L., Rajasree, K. P., Singhania, R. R., & Pandey, A. (2010). Bio-ethanol from water hyacinth biomass: an evaluation of enzymatic saccharification strategy. Bioresource technology, 101(3), 925-930.

[25] Shao, B., Han, Z., Pang, R., Wu, D., Xie, B., & Su, Y. (2023). The crystalline structure transition and hydrogen bonds shift determining enhanced enzymatic digestibility of cellulose treated by ultrasonication. Science of The Total Environment, 876, 162631.

[26] Stramarkou, M., Missirli, T. V., Kyriakopoulou, K., Papadaki, S., Angelis-Dimakis, A., & Krokida, M. (2023). The Recovery of Bioactive Compounds from Olive Pomace Using Green Extraction Processes. Resources, 12(7), 77.

[27] Yang, L., Huang, Y., Zhao, M., Huang, Z., Miao, H., Xu, Z., & Ruan, W. (2015). Enhancing biogas generation performance from food wastes by high-solids thermophilic

anaerobic digestion: Effect of pH adjustment. International Biodeterioration & Biodegradation, 105, 153-159.

[28] Pavi, S., Kramer, L. E., Gomes, L. P., & Miranda, L. A. S. (2017). Biogas production from co-digestion of organic fraction of municipal solid waste and fruit and vegetable waste. Bioresource technology, 228, 362-367.

[29] Kiran, E. U., Trzcinski, A. P., & Liu, Y. (2015). Enhancing the hydrolysis and methane production potential of mixed food waste by an effective enzymatic pretreatment. Bioresource technology, 183, 47-52.

[30] Lin, C. S. K., Pfaltzgraff, L. A., Herrero-Davila, L., Mubofu, E. B., Abderrahim, S., Clark, J. H., ... & Luque, R. (2013). Food waste as a valuable resource for the production of chemicals, materials and fuels. Current situation and global perspective. Energy & Environmental Science, 6(2), 426-464.

[31] Goyal, N., Gupta, J. K., & Soni, S. K. (2005). A novel raw starch digesting thermostable α-amylase from Bacillus sp. I-3 and its use in the direct hydrolysis of raw potato starch. Enzyme and Microbial Technology, 37(7), 723-734.

[32] Zhang, Q., He, J., Tian, M., Mao, Z., Tang, L., Zhang, J., & Zhang, H. (2011). Enhancement of methane production from cassava residues by biological pretreatment using a constructed microbial consortium. Bioresource Technology, 102(19), 8899-8906.

[33] Wu, Y., Hu, W., Zhu, Z., Zheng, X., Chen, Y., & Chen, Y. (2022). Enhanced volatile fatty acid production from food waste fermentation via enzymatic pretreatment: new insights into the depolymerization and microbial traits. ACS ES&T Engineering, 3(1), 26-35.