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ACIDIC-ENZYMATIC CO-TREATMENT OF FOOD WASTE FOR HIGH SUGAR RECOVERY AND BIOGAS PRODUCTION

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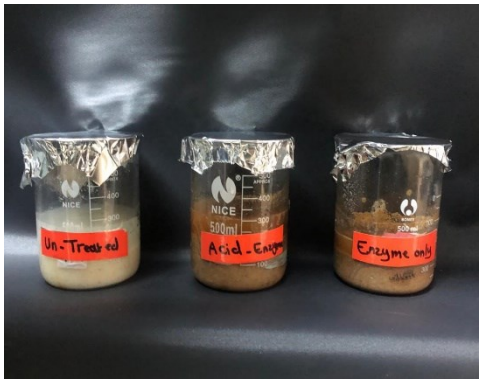
Graphical abstract



Abstract

Food-waste (FW) has a high content of fermentable sugar which can be exploited to generate an alternative source of fuel that could replace fossil fuels. Anaerobic digestion (AD) is an approach to convert organic-waste into high-value products like biomethane and hydrogen. However, hydrolysis is deemed to be a rate-limiting process in the AD process, limiting biogas production. This study seeks to improve the hydrolysis process through pre-treating FW with chemical and biological treatments. We show that the enzymatic treatment substantially improved the hydrolyzation and solubilization of food waste, resulting in a three folds increase in biogas production compared to untreated food waste. A co-treatment of biological and enzymatic treatments significantly improved the hydrolysis process, solids reduction, and solubilization of the substrate. The soluble chemical oxygen demand (SCOD) and reducing sugar were increased by 50% and 25% respectively, compared to enzymatically treated only. However, the inhibitory effect of accumulated salts from the treatment has limited the application of anaerobic digestion. Our results reveal that using enzymatic and acidic pre-treatment can significantly enhance hydrolysis of FW to increase biogas production, highlighting the potential of AD.

Keywords: Acidic-enzymatic treatment, biogas from food-waste, solid state fermentation enzyme, reducing sugar, biological treatment

1.0 INTRODUCTION

The projection of food waste is increasing in the current 25 years, particularly in Asian countries. Paritosh *et al.* [1] reported that there would be an increase from 278 to 416 million tonnes from 2005 to 2025. Food waste accounts for 23% of municipal waste, taking up to 30% of the total trash disposed into landfills and incinerators [2]. This problem has led to uncontrolled fermentation in landfills, emitting greenhouse gases, polluting groundwater, increasing disposal cost, and damaging incinerators by high-temperature fluctuation due to high water content. On the contrary, food waste has a high content of fermentable substrates such as sugars, fats, starches, lipids, proteins, and cellulose [3], which makes it an excellent substrate to produce high-value products (e.g., biofuels and platform chemicals) [4].

Anaerobic digestion is an approach to convert organic waste, such as food waste, into valuable products like biogas. During hydrolysis, complex organic matters like carbohydrates, protein, and fats are broken down into their monomers, reducing sugars, amino acids, and fatty acids, respectively.

Hydrolysis is deemed to be the rate-limiting process in anaerobic digestion; hence, the hydraulic retention time of the digester might take up to 1 month [5].

The constraints imposed by the structural and compositional features of food waste, which are the degree of polymerisation, crystallinity, lignin and pectin content, accessible surface area, and more, result in limiting the hydrolysis step of anaerobic digestion, hence increasing the hydraulic retention time [6]. It was reported previously that separating the hydrolysis from the remaining processes in an anaerobic digester could improve the digester's performance and reduce the hydraulic retention time [7]. Organic substrates can be hydrolysed using different types of treatments, including biological, chemical, thermal, and physical. For the chemical treatment, acid or alkaline are used for the cleavage of bonds and addition of water molecule. Alkaline treatment is used for the hydrolysis of proteins, lignin, and fats, whereas acidic treatment is used for the hydrolysis of carbohydrates like cellulose and starch [8].

For the biological treatment, hydrolytic enzymes break down complex substrates into their monomers, allowing a higher surface area to be attacked by the microbes, thus improving the digestion of lignocellulosic biomass in the system. Multiple hydrolytic enzymes are used in the pre-treatment process, such as Protease, Lipase, and Carbohydrase enzymes.

Moon *et al*. [3] studied the hydrolysis of kitchen food-waste to reduce sugar for the application of methane production using a mixture of the enzymes, carbohydrase, protease, and lipase. The study achieved a value of 9.1 g/L of reducing sugar. Another similar study was conducted by Moon *et al*. [9] on kitchen food-waste hydrolysis but for ethanol production. Interestingly, very high value of 164 g/L reducing sugar was achieved from the same substrate but using a different enzyme called glucoamylase.

Uçkun Kiran *et al*. [10] utilized fungal mash rich in hydrolytic enzymes produced by solid-state fermentation using cake waste for the enzyme production and methane production. The mash was rich in glucoamylase and protease and 89.1 g/L of reducing sugar was recovered.

Up to our knowledge, no studies have tested the effectiveness of acidic-enzymatic hydrolysis and biogas production in anaerobic digestion. The diluted acid treatment is to break down the polymer's structure to make it more susceptible to an enzymatic attack, hence improve the hydrolysis process and increase the amount of soluble substrate.

This study focuses on optimizing the enzyme to substrate ratio. The results of optimization process were used in studying the effect of acidic-enzymatic treatment of food-waste with different acid concentrations on the soluble chemical oxygen demand (SCOD), total solid reduction, the release of monomers, and biogas production.

2.0 METHODOLOGY

2.1 Overview of the Methodology

Two sets of experiments were conducted as shown in Figure 1. The first set of experiments started with the collection and preparation of food-waste, then the single enzymatic pre-treatment process was optimized to maximize production rate. Next, the co-treatment of food-waste with diluted sulfuric acid under different concentrations, followed by enzymatic treatment with the previously optimized conditions. Lastly, the effect of food-waste pre-treatment on the performance of anaerobic digesters will be monitored.

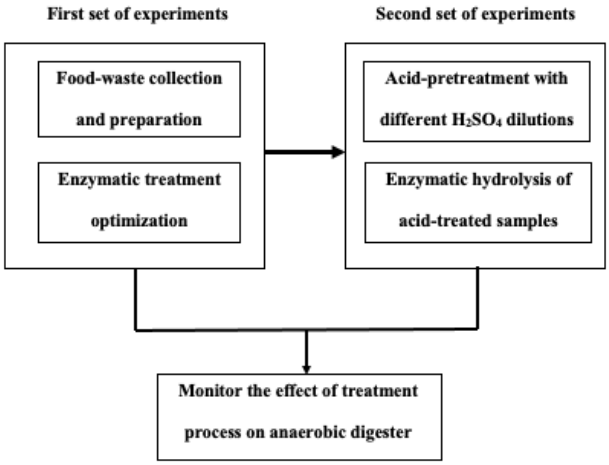


Figure 1 Flowchart of methodology diagram

2.2 Preparation and Characterization of Food-Waste

Food waste was collected from IIUM. Bones were removed and food-waste was homogenized to reduce particle size. Next, the total carbohydrate s [11], total protein [12], total lipid [13], total solids, volatile solids, chemical oxygen demand and moisture of the samples were calculated based on standard method. The samples were kept under -20°C until further use.

2.3 Optimization of Enzymatic Hydrolysis

Food-waste was hydrolysed using a cocktail of natural enzymes produced and provided by IIUM lab. The enzymes were produced by solid state fermentation of food-waste. The main enzymes, namely cellulase, amylase, protease, and lipase were identified and extracted as explained in a previous study by Sonia and others [14]. Since the main constituent of the food-waste is starch, the reaction's pH was fixed at 7, which is amylase's optimum pH value. The optimum parameters for food-hydrolysis were determined by face-centered central composite design (FCCCD) of response surface methodology (RSM) using Stat-Ease

Design-Expert v13. The experimental runs are shown in Table 1. The two tested factors were enzyme loading between 4-10 (w/w) and substrate concentration between 4-10 (TS%). The design responses are the amount of reducing sugar and FAN released.

2.4 Co-treatment: Acidic-enzymatic

Food-waste is first treated with different concentrations of 18 M sulfuric acid (0.5,1, 1.5 %) (v/v) for 1 h at room temperature, then pH is adjusted to 7 followed by enzymatic treatment at 50°C for 16 h. The release of reducing sugars was quantified using DNS method. Free amino nitrogen was measured using ninhydrin reagent.

2.5 Biogas Production

The hydrolysed food-waste fed into a 500 ml anaerobic digester was set up for biogas production, initial pH of 7, and temperature of 50°C. The digester was inoculated with 10%(v/v) of anaerobic sludge collected from Sime-Darby Research Centre at Carey Island, Malaysia [15]. FW- hydrolysate was diluted with water 1:2 ratio respectively. The digester was fed with 50 ml/day of treated food-waste and operated for 5 days. Biogas volume was monitored daily prior to feeding. The final pH was also monitored.

2.6 Analytical and Statistical Analysis

Total solids was measured by drying a known volume of sample at 120°C for 3 h, then the final weight was measured. Similarly volatile solids were measured by placing the same sample in the furnace at 550°C for 20 min, final weight was measured. Moisture was measured by substracting the final total solid weight from the initial sample weight [16]. Total carbohydrates was measured using anthrone reagent [11], total proteins using Bradford reagent [17], lipids using modified folch extraction [13]. Reducing sugar was measured using DNS method [18]. Free amino nitrogen was measured using ninhydrin reagent [19], SCOD was measured using Hach digestion vials.

3.0 RESULTS AND DISCUSSION

3.1 Food-waste Characterization

Food-waste was analyzed as previously described. The substrate had high content of rice, followed by equal amounts of meat and vegetables. Hence high value of carbohydrates, followed by lipids and proteins. The table below shows the characteristics of food-waste obtained:

Table 1 Food-waste characterization

Food-waste characterization	Value
Total carbohydrates (g/L)	66.5
COD (g/L)	112.5
TS%	30
VS%	96.5
Moisture%	70
Total proteins g/L	16
Total lipids g/L	9.55
rice %	70
vegetables %	15
proteins %	15

3.2 Optimization of Enzymatic Treatment

The optimum condition and interaction of the factors enzyme loading and substrate concentration were determined using RSM. At central point conditions (6%(w/v) enzyme loading and 8% (w/v) substrate concentration). The maximum reducing sugar release of 14.88 g/L was achieved at an enzyme loading of 8%(w/v) and substrate concentration of 10%. Analysis of variance (ANOVA) was performed and the results are described in Table 2. The P-value was 0.0019 ($P<0.05$), and F-value 12.37 which indicates that the terms were significant. Lack of fit value is 0.2945 (>0.005) which is not significant. The model, and models A, B, A^2 were also significant. However, models B^2 and AB were not significant. These models terms could not be excluded to assist the structure of the model.

Based on the regression analysis, the best model for the relation of reducing sugar (Y) with enzyme loading(A) and substrate concentration (B) is fitted in the equation below:

$$Y= 11.82+2.29A+1.88b- 0.38AB-1.56A^2 +0.98B^2$$

Table 2 ANOVA results for reducing sugar release

DF	Sum of Square	Value	Mean Prob > F	F		Source	Squares
	Model	61.87	5	12.37	13.09	0.0019	significant
	A	*31.37*	*1*	*31.37*	*33.19*	*0.0007*	
	B	*21.21*	*1*	*21.21*	*22.44*	*0.0021*	
	A^2	*8.67*	*1*	*8.67*	*9.17*	*0.0192*	
	B^2	*1.63*	*1*	*1.63*	*1.72*	*0.2305*	
	AB	*0.59*	*1*	*0.59*	*0.63*	*0.4543*	
	Residual	6.62	7	0.95			
	Lack of Fit	*3.76*	*3*	*1.25*	*1.75*	*0.2945*	*not significant*
	Pure Error	*2.86*	*4*	*0.71*			
	Cor Total	68.49	12				

The actual values of the treatment align linearly with the predicted values (Figure 2), which shows another evidence of the successfulness of the model. Theoretically increasing the enzyme loading would yield high reducing sugar values. The optimization results showed that the highest enzyme concentration of 8%(w/v) did not show major effect in increasing the reducing sugar yield percentage substantially (Figure 3). Although the maximum sugar release was observed in samples with an enzyme loading of 8%(w/v) with substrate concentration of 10% with value of 14.88 g/L. Samples with the same substrate concentration with a lower enzyme loading of 6%(w/v) showed similar results, with a sugar release of 14.1 g/L. At a lower substrate concentration of 6%(w/v), treating with enzyme loading of 6% or 8%(w/v) did not lead to a substantial effect on releasing reducing sugar.

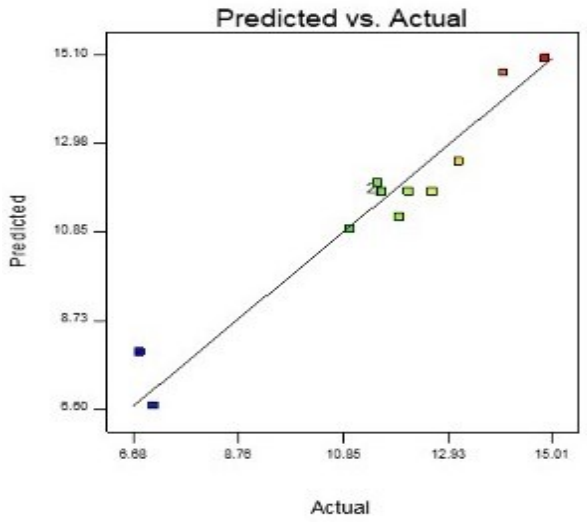


Figure 2 Predicted vs actual value of reducing sugar release

On the other hand, increasing the enzyme loading while maintaining the substrate concentration had a negative effect on the final reducing sugar release (Figure 3). The hypothesis explaining this is related to the medium viscosity. Adding high enzyme loading increases the amount of solids in the medium, which in return increases the viscosity. Viscosity plays a crucial role in mixing. Higher viscosity reduces the mixing efficiency. Mixing is very crucial for the enzyme substrate interaction, as a lower mixing efficiency decreases substrate-enzyme interaction. This results in a lower hydrolysis rate, and a lower amount of reducing sugar is released. Since the main aim of the study is to maximize the substrate loading while minimizing the enzyme loading, enzyme loading of 6%(w/v) and substrate concentration of 10% was used for future experiments.

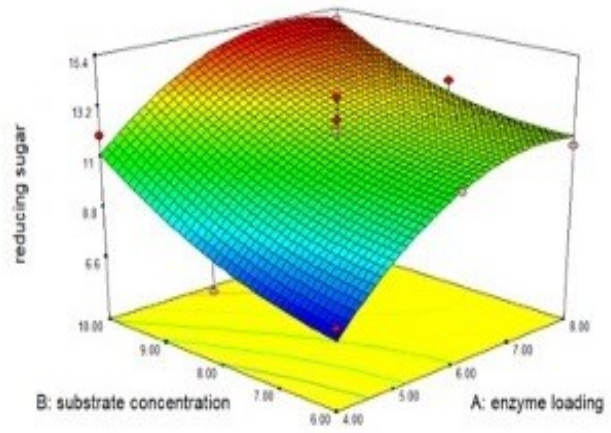


Figure 3 3D surface of the interaction between substrate concentration and enzyme loading

3.3 Acidic-enzymatic Co-treatment

Although the enzymatic treatment of food waste was successful, the substrate conversion only accounted for 35% of the substrate's total volume (Figure 3). As observed from previous results, increasing the enzyme loading had no substantial effect on the treatment, hence, the pre-treatment should be improved to increase the conversion percentage. Using diluted acid treatment breaks down the polymer's structure to make it more susceptible to an enzymatic attack. Based on previous experiments, substrate concentration of 10%(w/v) with enzymatic loading of 6% is used for the co-treatment.

3.4 Total Reducing Sugar

The acid-enzyme pre-treatment of food-waste has substantially improved the release of reducing sugar compared to control and samples treated with enzymes only. Samples pre-treated with 0.5%(v/v) of concentrated acid followed by enzymatic hydrolysis has shown an improvement in the release of reducing sugar by 49.2% and 256% compared to samples treated with enzyme only and without enzymes respectively (Figure 4). However, the release of sugar decreased with the increase in acid concentration. Samples treated with 1.5%(v/v) of acid followed by enzyme showed a drop by 16% compared to samples treated with enzyme only (Figure 4). The decrease of reducing sugar with the increase of acid concentration could be due to two reasons:

i. higher concentrations of acid dehydrates glucose under high and room temperature [20]. The Sulfuric acid dehydration of glucose equation is as follow:

$C_6H_{12}O_6$ (glucose) + H_2SO_4 (sulfuric acid) → 6C (carbon) + $6H_2O$ (water) + H_2SO_4 (sulfuric acid)

Sulfuric acid slowly and gradually dehydrates glucose into carbon and water; thus, it is advised to reduce the total treatment time with higher acid concentration.

ii. The sample's pH was neutralized prior to the enzymatic treatment. During the neutralization, Na_2SO_4 salts are produced along with water which increases the ionic strength of the sample. An increase in the ion strength affects the stability of the enzyme, leading to lower enzyme activity [21].

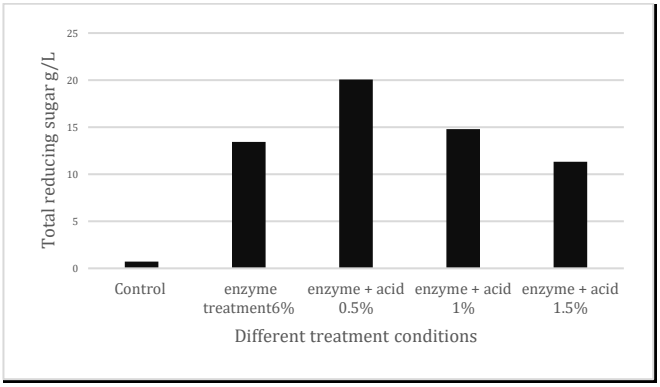


Figure 4 Effect of enzymatic treatment and different acidic-enzymatic treatment (0.5, 1, 1.5%)**(v/v)** total reducing sugar release

3.5 Total Free Amino Nitrogen

The hydrolysis of proteins decreased with acid pre-treatment by 26% compared to samples treated with enzymes only (Figure 5). Low pH value has a negative impact on the three-dimensional structure of proteins. Acidic pH changes the attractions between the side chain groups of the protein, owing to the high concentration of hydrogen ions in the acidic medium[22]. Denatured proteins lose their original folded shape, and this new protein structure does not bind to the enzyme's active site for hydrolysis, which explains the reduction of amino acids recovered. The increase of acid concentration had no substantial effect on the free amino nitrogen release, the FAN values were relatively close to all three samples treated with different concentrations of acid (Figure 5).

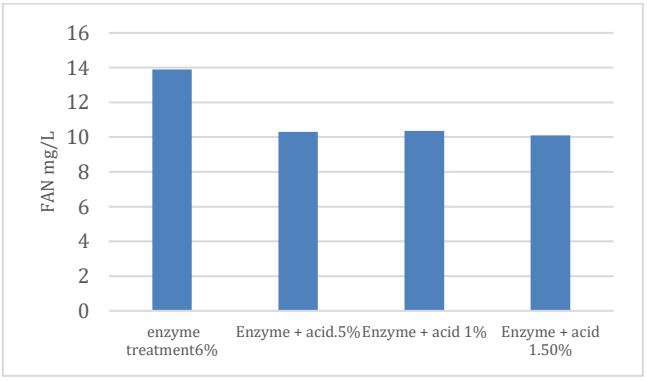


Figure 5 Effect of enzymatic treatment and different acidic-enzymatic treatment (0.5, 1, 1.5%) (v/v) total free amino nitrogen release

3.6 Total Solids

There was a noticeable improvement in the reduction of solids with enzymatic hydrolysis, and enzyme-acid hydrolysis by 24% and 34% respectively (Figure 6). However, increasing the amount of acid over 0.5%(v/v) has reduced the overall hydrolysis efficiency. As explained previously, high concentration of salts affects the enzymatic activity. In addition, lower pH denatures proteins. This reduces the release of FAN, since denatured proteins cannot be hydrolyzed and are insoluble due to their change of structure.

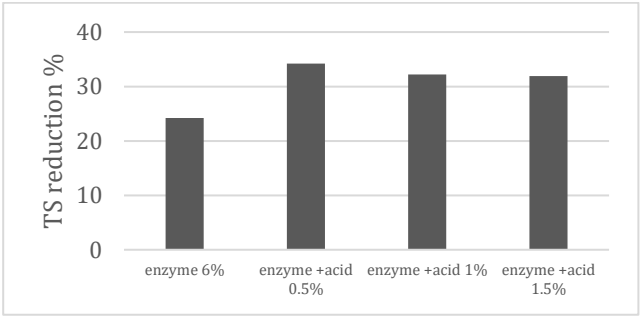


Figure 6 Total solids reduction of enzymatic treatment and different acidic-enzymatic treatment (0.5, 1, 1.5%)

3.7 Soluble Chemical Oxygen Demand

The increase of SCOD values with the acid pre-treatment indicates the effectiveness of the co-treatment. The SCOD has increased by 104.5% in samples pre-treated with 0.5%(v/v) of acid concentration (Figure 7). Increasing the acid concentration showed an improvement in solubilizing the organic matter compared to enzymatic treatment alone but samples treated with low acid showed the best condition for solubilizing organic matter. Neutralizing the pH after the acid treatment prior to enzymatic treatment, increases the number of salts in the mixture. This increases the ions concentration, which affects enzymes activity [21].

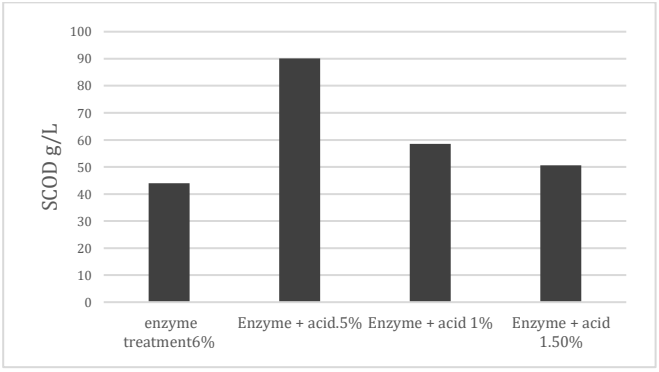


Figure 7 Soluble chemical oxygen demand of enzymatic treatment and different acidic-enzymatic treatment (0.5, 1, 1.5%)

3.8 Biogas Production

Anaerobic digester was set up and seeded with 10%(v/v) of anaerobic sludge. To check the enhancement of the pre-treatment of food-waste, the digester was fed with different food-waste under different cycles. On the first cycle the digester was fed with enzymatically hydrolyzed Food-waste. On the first feeding, digester was fed with FW diluted with distilled water at a ratio of 1:2 respectively, the digester's pH was adjusted to pH7. The biogas production was as follows:

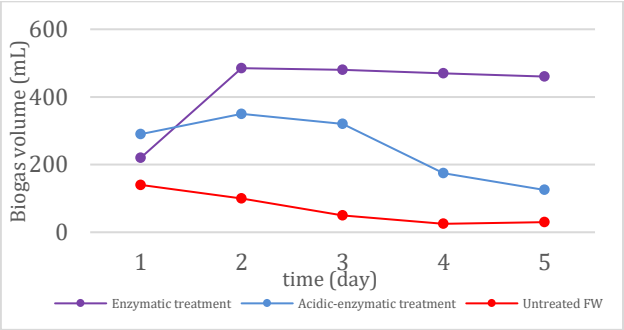


Figure 8 Biogas production of digesters: enzymatic treatment, acidic-enzymatic treatment, untreated food-waste

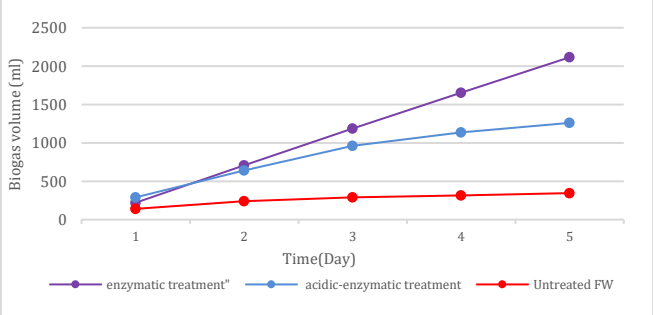


Figure 9 Cumulative biogas production of digesters: enzymatic treatment, acidic-enzymatic treatment, untreated food-waste

The daily biogas production for reactors for enzymatically treated FW, acidic-enzymatic treated FW, and untreated FW were monitored for 5 days. Digesters fed with enzymatically treated FW showed the best performance in the daily and cumulative production of biogas, by 91% and 600% compared to acidic-enzymatic treated FW, and untreated FW respectively (Figures 8 & 9). The enzymatic treatment has increased the amount of the soluble sugars in the feed, resulting in an increase in the biogas production. Similar studies reported the effectiveness of enzymatic pre-treatment. Speda *et al.* [23] reported that enzymatic treatment has enhanced the degradation of lignocellulose and improved the daily biogas production.

Although the soluble oxygen demand and amount of reducing sugar released was higher in the acidic-enzymatic co-treatment, the cumulative biogas production of the digester was substantially lower compared to enzymatically treated FW digesters (Figure 9). The pH drops drastically during acidic treatment of FW, which was then adjusted to pH7 for the enzymatic treatment, producing Na_2SO_4 salt and water. High salinity mainly included cations of Na, K, Ca, Mg, and Fe, which could hinder the Anaerobic digester seriously and dehydrate cell walls through the action of osmosis, hence, disrupts the biogas producing microbes [24].

4.0 CONCLUSION

The enzymatic treatment has shown a substantial improvement in hydrolyzing and solubilizing food-waste, hence enhancing the biogas production compared to untreated food-waste. Although the co-treatment has shown significant improvements in the hydrolysis process, solids reduction, and solubilizing the substrate, the inhibitory effect of accumulated salts from the treatment has limited the application of anaerobic digestion.

Enzymatic-treatment has shown the best rate and volume of biogas production over acidic-enzymatic treatment. Thus, acidic-enzymatic treatment could be explored for applications that are not affected by the salinity of the substrate.

Conflicts of Interest

The author(s) declare(s) that there is no conflict of interest regarding the publication of this paper.

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