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Anaerobic Digestion for Biomethane Production from Food Waste Pretreated by Enzymatic Hydrolysis

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Abstract

Food waste (FW) is one of the main problems in the world due to the continuous increase in the global population. Anaerobic digestion (AD) of FW was an alternative and economical solution to develop an effective method to enhance biomethane (BioM) production that uses enzyme pretreatment and hydrolysis of FW by locally produced cellulase and amylase enzymes. In this study, two types of sources fungi (TNAF-1 to TNFA-3) and (TNBC-1 to TNBC-3) strains were isolated from animal feed and compost. The cellulase and amylase activities were 300U/mL & 400U/mL, respectively. Based on OFAT results obtained, optimization of three factors such as pH of 5, TS of 12.5% (V/V) and enzyme loading of 80U/mL was carried out by applying the FCCCD under the RSM to develop a second-order regression model successful improvement in the production of reducing sugar of 162mg/mL was achieved. However, biogas yield was optimum using OFAT parameters such as the biogas inoculum of 25%, pH of 7, AD digestion times of 29 days, 500mL of hydrolysate food waste and room temperature at 30°C (±2). The results show the biogas contained was found such %3 hydrogens, 57% methane and 40% carbon dioxide. The new fungi are very potential for upgrading the biogas of BioM that is non-toxic as well as biodegradable, and therefore may be encouraging to the water treatment plants in future applications.

Keywords: AD systems; Food waste; Enzymes; OFAT; FCCCD; BioM.

1. Introduction

In recent years, food waste refers to the discarding of edible food, typically as a result of overproduction, spoilage, or consumer behavior. Food waste was a global issue that has significant economic, environmental, and social consequences [1]. According to the United Nations, approximately one-third of all food produced in the world was wasted, which translates to 1.3 billion tons of food annually [2]. In developed countries, a significant portion of food waste occurs at the consumer level due to food spoilage, confusion over expiration dates, and overbuying. In developing countries, much of the food waste occurs at the production and processing stages due to inadequate infrastructure, poor storage facilities, and inefficient supply chains [3].

The economic impact of food waste was significant, with an estimated cost of $940 billion per year [4]. This cost includes the value of wasted food, as well as the costs associated with disposal, such as transportation, processing, and landfill fees. Food waste also has serious environmental consequences, as it was a major contributor to greenhouse gas emissions. When food decomposes in landfills, it produces methane, a potent greenhouse gas that contributes to global warming. Additionally, the resources used to produce the wasted food, such as water and energy, are also wasted. Addressing food waste was crucial to achieving sustainable development and reducing the negative impact of food production on the environment. Strategies to reduce food waste include improving storage and transportation infrastructure, educating consumers on proper food handling and storage, donating excess food to those in need, and implementing policies and regulations to reduce waste at the production and processing stages [5].

There are several types of food waste processing methods that are commonly used to manage food waste. These include composting, anaerobic digestion, and incineration. Each of these methods has its own advantages and

disadvantages, and the choice of method depends on several factors, including the amount of waste generated, the type of waste, and the available resources [6]. Composting was a process in which organic waste was decomposed under controlled conditions to produce compost, a nutrient-rich soil amendment. Composting can be done in small-scale backyard composting systems or in large-scale industrial composting facilities. Composting was a popular method for managing food waste because it was relatively inexpensive and produces a useful end product. However, composting can be time-consuming and may require a significant amount of space. Anaerobic digestion was a process in which organic waste was broken down by microorganisms in the absence of oxygen to produce biogas, a mixture of methane and carbon dioxide [7]. Biogas can be used as a renewable energy source, and the byproduct of anaerobic digestion, called digestate, can be used as a fertilizer. Anaerobic digestion was a popular method for managing food waste in large-scale facilities, such as food processing plants or wastewater treatment plants. However, anaerobic digestion requires a significant investment in infrastructure and may not be feasible for small-scale operations. Incineration was a process in which waste was burned at high temperatures to produce energy. Incineration was a popular method for managing food waste in some countries because it can generate electricity or heat. However, incineration was often criticized for its potential to release harmful pollutants into the air, and it can be expensive to build and operate. Each food waste processing method has its own unique advantages and disadvantages, and the choice of method depends on several factors, including the amount of waste generated, the type of waste, and the available resources [8]. Composting was a relatively inexpensive method that produces a useful end product, while anaerobic digestion can generate renewable energy and a useful fertilizer.

Anaerobic digestion was a process by which organic material was broken down in the absence of oxygen to produce biogas, a mixture of methane and carbon dioxide, and a nutrient-rich residue called digestate. There are several different methods of anaerobic digestion, including: Batch digestion: This was the simplest method of anaerobic digestion and involves loading a tank with organic material, sealing it, and allowing the digestion process to occur [9]. Once the process was complete, the biogas and digestate were removed, and the tank was refilled. Continuous digestion method, organic material was continuously added to the digester while biogas and digestate were continuously removed. This method was often used in large-scale industrial operations. Two-stage digestion method involves separating the hydrolysis and methanogenesis stages of the digestion process into two separate tanks [10]. The hydrolysis tank breaks down the organic material into simpler compounds, while the methanogenesis tank converts these compounds into biogas. Plug-flow digestion method involves a long and narrow tank that allows organic material to flow through the tank at a constant rate, allowing for more efficient digestion [11]. Upflow anaerobic sludge blanket (UASB) method uses a vertical reactor that separates the biogas and liquid phases using a sludge blanket, allowing for more efficient digestion and removal of biogas [12]. Anaerobic membrane bioreactor (AnMB) method uses a membrane to filter out the solids in the digester, allowing for more efficient digestion and better separation of the biogas and liquid phases.

Food waste converted into biogas through the process of anaerobic digestion was a sustainable and eco-friendly solution for waste management. Anaerobic digestion was a biological process that occurs when organic materials are broken down by microorganisms in the absence of oxygen. This process converts the organic matter into biogas, which was a mixture of methane and carbon dioxide, as well as a nutrient-rich slurry. However, anabolic digestion was a type of anaerobic digestion that occurs at a pH level between 6.5 and 8.0. It was also known as the mesophilic process, as it takes place at a temperature of around 35-40°C [13]. During anabolic digestion, the microorganisms break down the complex organic matter in the food waste, such as carbohydrates, proteins, and fats, into simpler compounds. This process was exothermic, which means it generates heat and can therefore be self-sustaining. Moreover, the biogas produced from anabolic digestion can be used to generate electricity or heat, or can be upgraded to biomethane and used as a vehicle fuel. The nutrient-rich slurry left over from the digestion process can be used as a fertilizer or soil conditioner, returning valuable nutrients back to the soil. On the other hand, the process of anaerobic digestion offers many benefits compared to traditional waste management methods. By diverting food waste from landfill, it helps to reduce greenhouse gas emissions and prevents the release of methane, a potent greenhouse gas, into the atmosphere. It also provides a sustainable source of energy that can help to reduce our dependence on fossil fuels. In addition, the nutrient-rich slurry produced during the process can be used to improve soil quality and support sustainable agriculture. In addition, converting food waste into biogas through the process of anabolic digestion offers a sustainable and eco-friendly solution for waste management [14].

In this paper, biogas production from food waste using enzymatic hydrolysis was a process that involves breaking down the complex organic compounds in the food waste into simpler molecules, which can then be used by anaerobic bacteria to produce biogas. Collect and prepare the food waste from restaurants, supermarkets, and households was used as the substrate for biogas production. The food waste was sorted and shredded to increase the surface area for enzymatic hydrolysis. The food waste was treated with enzymes to break down the complex organic compounds into simpler molecules such as sugars, amino acids, and fatty acids. This process was called enzymatic hydrolysis and was typically carried out in a reactor tank under controlled conditions of temperature and pH. However, the hydrolysate produced in step 2 was then transferred to an anaerobic digester where anaerobic bacteria digest the hydrolysate to produce biogas. The digester is a closed vessel where the temperature, pH, and mixing conditions are optimized for bacterial growth and biogas production. In addition, the biogas produced in the digester contains methane, carbon dioxide, and other impurities. It needs to be purified before it can be used as a fuel. The efficiency of biogas production depends on several factors, including the composition of the food waste, the type of enzymes used for hydrolysis, and the operating conditions of the reactor and digester. Optimization of these factors was crucial for maximizing the biogas yield and reducing the cost of production. Finally, this was done using a gas

scrubber or purification systems. The purified biogas was used as a fuel for heating, electricity generation, or as a transportation fuel.

2. Material and Method

2.1. Sample (FW) Collection and Characterization

Food waste was collected from restaurant and super shop in Bangladesh. The sample of FW was kept in a cold room (4°C) to prevent the growth of fungus on it. The main raw material used in this study was domestic food waste collected from different canteens in Dhaka, Bangladesh. Collected food waste was mixed food around 30 kg composed of 10 kg of mixed vegetable, 5kg of mixed fruits, 5 kg of cooked rice, 5 kg of leftover, 3 kg of meat and fish and 2 kg peel and others. The second raw material was anaerobic sludge collected from local Dasherkandi Sewerage Treatment Plant, Dhaka, in Bangladesh and used as an anaerobic digester inoculum. According to different eating habits, food waste composition was varied. Therefore, a consistent source of food outlets was selected for food collection and characterization. The main parameters such as pH, volatile solids, carbohydrates, protein, fat and oil, total solids and mineral compositions was considered to the high content of BioM production. All parameters were analyzed using established and standard methods used [15].

2.2. Inoculum Preparation for Hydrolysis Enzyme

The method described by Alam, *et al.* [16] was used to prepare starter culture for enzyme synthesis. To preserve the constancy of colony percentage, each seven-day-old fungal cultured plates was rinsed with about 25 mL sterilized distilled water using a bent glass rod. To separate the mycelia from the extract solution, the isolated fungal samples were screened utilizing Whatman No. 1 filter paper. When quantifying the concentration (1.5×10^8 to 3×10^8 spores/mL) with a Hemocytometer, the supernatant was placed into a 150 mL Erlenmeyer flask and utilized as spore suspension.

2.3. AD based Enzymatic Hydrolysis Process

The enzymatic hydrolysis was carried out in a 10L plastic container where the pretreatment was done. The activity 326 CMC U/mL of cellulase and 400U/mL of amylase enzymes were used from varying does 20U to 200U/50mL of pretreated food waste to evaluate the rate of enzymatic hydrolysis. In this step, initial cellulase enzyme dose for 5days (40 to 140 U), enzyme pH (4.5 to 7), hydrolysis time (0 to 5 days) and enzyme dose (40 to 200 U) was observed to optimize the process condition. The flasks were shaken at 0 rpm at room temperature 30°C (±2). After 24 hours, samples were collected and centrifuged at 5000 rpm for 20 minutes, and the supernatant was examined for TS, VS and COD. Besides substrate concentration, cellulase and amylase enzymes dose were also to be optimized by FCCCD.

2.4. Optimization of Enzymatic Hydrolysis

Anaerobic digesters contain organic matters that consist of complex polymers which are not accessible to microorganisms without being further broken down by hydrolysis pretreatment or other pretreatments. Consequently, the process of hydrolysis assists the purpose of biodegrading organic macromolecules into its smaller components, which in sequence can be used by acidogenic bacteria and facilitate methanogenesis step. However, it has been mentioned earlier in the literature review that the hydrolysis of food waste anaerobic digestion is still a rate-limiting step in its biological processes of anaerobic digestion. To overcome this rate limit, food waste hydrolysis was investigated by using hydrolytic enzyme in hydrolysis reactor. This is because of enzyme that are able to convert carbohydrates, lipids, and protein into sugar, long chain fatty acids and amino acids respectively as explained by Li, *et al.* [17]. After enzymatic cleavage, the products of hydrolysis are able to diffuse through the cell membranes of acidogenic microorganisms and facilitate biodegradation of organic compound. Therefore, it is important to add enzymes that can enhance hydrolysis and facilitate the degradation of the complex carbohydrate. Therefore, the effect of the enzyme amounts and biodegradation time on the hydrolysis of food waste was examined to estimate the feasibility of using enzyme in hydrolysis process for the pretreatment of food waste and find the optimum of the amount of enzyme and the time of biodegradation in hydrolysis process. Optimization of the enzymatic hydrolysis was done in two steps. In the first step, three important parameters were observed in a OFAT design to evaluate possible optimum levels of the parameters. The parameters were hydrolysis pH, hydrolysis time, and enzyme dose with the hydrolysis treatment strategy of the food waste established in the previous sections. In the second step, the parameters found to be optimum from the OFAT study were further observed by the statistical optimization method, FCCCD.

3. Results and Discussion

3.1. Characterization of Food Waste

Table 1 shows the properties of the food waste composition utilized in this research. After removing the bones and shells, the remaining waste was blended with distilled water in a 1: 1 (w/w) ratio. Generally, characterization of food waste is very important before starting the optimization because it was used as a substrate during the media and process optimization for BioM production. From the different reports of characterization, it was observed that the factors were differed between several production units and other reasons. So, the current study of food waste

characterization was carried out before starting the important optimization study. It was observed from the results that the raw sample consists 15.6 % w/v of TS, 3.37 % w/w of cellulose and pH of 5.35, respectively.

Table-1. Characterization of the food waste

Parameters	Units	Concentration
Total Solids (TS)	(% w/v)	15.6
Total Suspended Solid (TSS)	(%w/v)	13.2
Total Dissolved Solids (TDS)	(%w/v)	2.4
Volatile Solids (VS)	(%w/v)	13.62
Volatile Suspended Solids (VSS)	(%w/v)	11.15
Volatile Dissolved Solids (VDS)	(%w/v)	2.47
Moisture Contain	(%)	79.53
pH	-	5.05
Chemical Oxygen Demand (COD)	g/L	89.2
Cellulose	% (w/w)	3.7
Hemicellulose	% (w/w)	1.19
Lignin	% (w/w)	0.57

Table 1 shows that the characterisation of the FW used was consistent with the composition of true house FW samples published in the literature [18-20]. Strazzera, *et al.* [21], observed 111 mg/mL of COD even though they collected the sample from the same house hold food waste. According to Lin, *et al.* [22], the majority of biodegradable wastes are dumped in landfills or utilized for first generation recycling and reuse such as organic manure and biofuels or livestock feed (where possible), resulting in both social and environmental effects observed to pollutant emissions to soil, air, and water. It is predicted that each and every ton of biodegradable garbage landfilled emits 4.2 tons of carbon dioxide (CO_2), accounting for 3% of total world greenhouse gas (GHG) emissions [23]. It was combined with water in a 1:1 (v/v) ratio and crushed into minute particles with a decrease the effect. It was then digested at 55°C for 12 hours, and a hydrolysate with a high sugar content (164 g/L) was produced [24-26].

3.2. One-Factor-At-A-Time (OFAT) Experimental Analysis

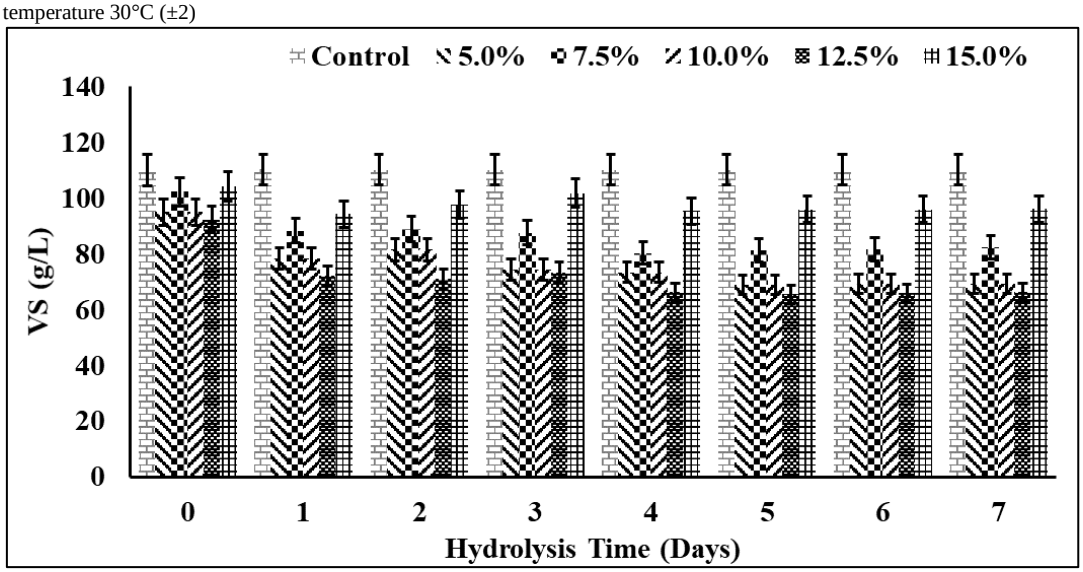
OFAT experimental analysis was further used to investigate the most contributing factors from the hydrolysis treatment process for the TS and VS values close to each other. This was conducted to obtain optimum design conditions for the TS and VS value prior the optimization stage [27]. The influential parameters subjected to OFAT studies consisted of enzyme pH, hydrolysis time and the enzyme dose in order to explore their effects on TS, VS and COD values close to each other.

3.2.1. Effect of Hydrolytic time on Hydrolysis Process

Proteins suffer denaturation and degradation of catalytic activity over time. Besides, some enzymes might be noticeably unstable and lose its activity over certain incubation time. This optimized process condition was observed through the degradation of organic compounds from 0 to 7 days of enzymatic hydrolysis. The effect of incubation time on degradation of organic compounds was studied, in which the degradation of organic compounds was determined every day up to 7 days. It was found that incubation time influenced degradation of organic compounds, where maximum degradation of organic compounds was observed after 5 days of incubation time with 71.0 g/L. The results in the examination of hydrolysis time show in Figure 1 suggested that the degradation of organic compounds was not increased by extending the incubation time after 7 days.

From Figure 1, VS was 110 g/L before hydrolysis and it was decreased to 92.35 g/L, 72.15 g/L, 71.05 g/L, 73.32 g/L, 66.30 g/L, 65.51 g/L, 65.98 g/L and 66.21 g/L for 0-day, 1 day, 2-days, 3-days, 4 days, 5 days, 6 days and 7 days respectively during the enzyme dose of 80U/mL and TS of 12.5%. The food waste characteristics used were determined before and after digestion. For the substrate with VS of 119.5 g/L before digestion, VS have decreased to 85.38 g/L respectively. Food waste with VS concentration of 90.4 g/L have reduced to 55.64 g/L. As a result, VS of the food waste have decreased due to the effect of the time and this express the facilitation of the degradation of the organic compound based on the decrease of VS.

Figure-1. Effect in VS of food waste hydrolysis with different incubation time and %TS. Other factors were fixed enzyme dose 80U/mL, pH 5 and room temperature 30°C (±2)

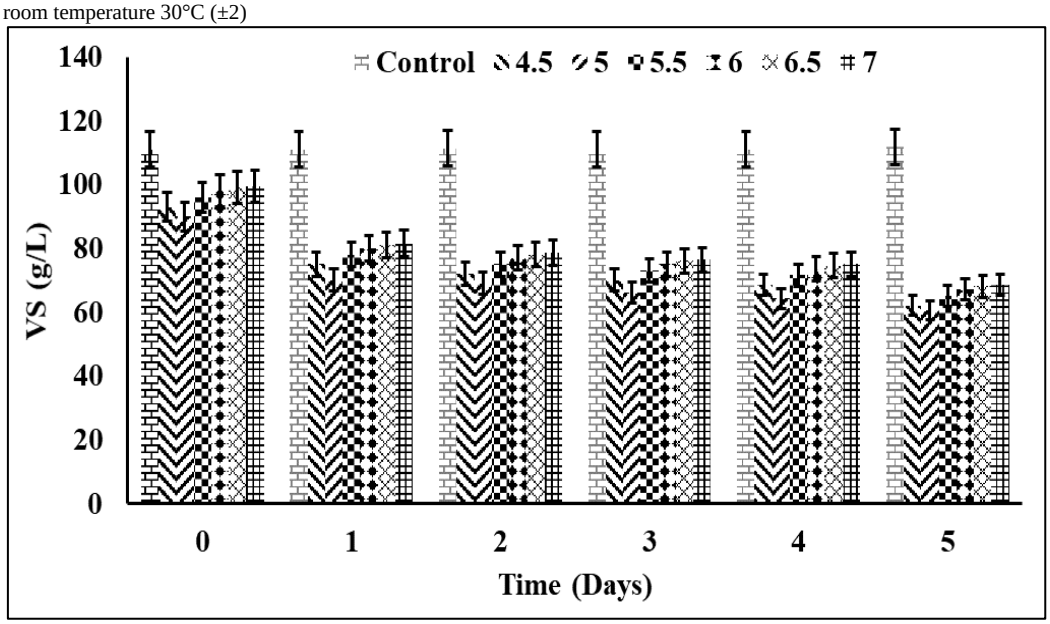


Concerning the optimum hydrolysis time, time had no influence on the organic substance, it was discovered as the different hydrolysis time gave VS values close to each other. This means that even duration of hydrolysis time can help organic compound to be degraded and improve hydrolysis of the food waste on the other hand, a control with 0 day which means food waste with no duration time was also studied to see the effect of hydrolysis time on food waste hydrolysis. From the values of VS of the control it is obvious that there is no decreased and there was no degradation of organic compounds in hydrolysis of food waste. The maximum VFA production of 0.71 and 0.72 mg COD/mg COD were obtained by BSA-dextran and amino acids-glucose fermentation, respectively, at Car/Pro ratios of 1 [28]. After 7 days of hydrolytic acidification, the volatile solids were reduced by 48.1%, yielding 6.8 g/L of volatile fatty acids and 4.7 g/L of acetic acid [29]. According to enzyme digestion research, growth substrates account for 40% of overall manufacturing costs, which can only be decreased by adopting a low-cost replacement substrate [30].

3.2.2. Effect of Hydrolytic pH on Hydrolysis Process

The effect of the hydrolysis pH on the degradation of organic compounds of the hydrolytic enzyme was observed at different pH ranging from pH 4.0 to 6.0 as shown in Figure 2. The optimal pH of this enzyme was established to be pH 5. At this pH, the degradation of organic compounds showed solubilization of 12.5%, whereas the control had 111.79 g/L of VS. Though the optimum degradation of organic compounds was found at pH 5, it was seen that the enzyme was reacted degradation of organic compounds within the range of pH from 4.5 to 7. The degradation of organic compounds at pH 4.5, 5, 5.5, 6, 6.5 and 7 were below from the optimum degradation of organic compounds VS by 62.03 g/L, 60.51 g/L, 65.03 g/L, 67.05 g/L and 68.53 g/L respectively. Other factors were fixed enzyme dose 80U/mL, incubation time 5 days, TS 12.5%, and room temperature 30°C (±2). However, the concentration of total dissolved solids (TDS) was increased slightly after the enzymatic hydrolysis, which showed the progression of hydrolysis and degradation of the complex organic matters present in the AD system.

Figure-2. Effect in VS of food waste hydrolysis with different pH. Other factors were fixed enzyme dose 80U/mL, incubation time 5days, TS 12.5%, and room temperature 30°C (±2)



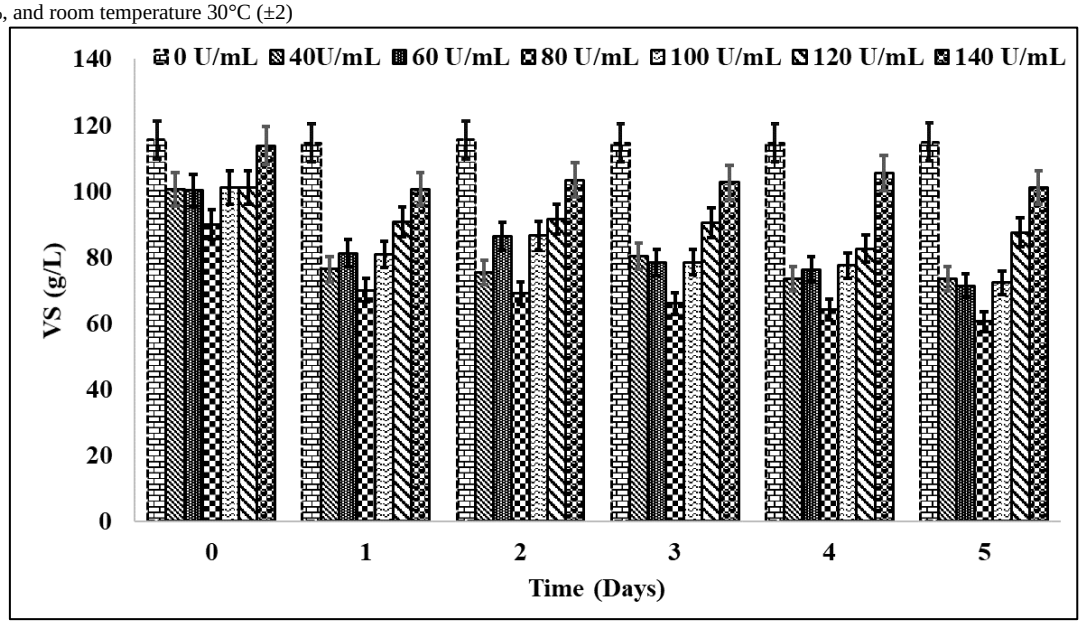
In the hydrolysis of this study, the soluble VS have increased from 60.25 g/L to 68.50 g/L which showed also a significance trend. On the other hand, TDS has increased sharply during the enzymatic hydrolysis time. Such increase is explained by the fact that when organic matters are degraded by hydrolytic enzyme, there is more dissolved organic and inorganic solid in the suspended form. pH [31], was operational factor that may be tuned to increase hydrolysis and acidogenesis rates. Furthermore, bacteria species and biochemical variation with pH has been found [32]. As a result, pH control was a promising in situ strategy for specific carboxylate synthesis in leach bed reactors.

3.2.3. Effect of Hydrolytic Enzymes does on Hydrolysis Process

The effect of biofilm amount was studied to find out the optimum volume of enzymes does that can give best condition for hydrolysis of the collected food waste. For that, different does of enzymes attached on the different mass on food waste were used. For each does of enzymes, TS and VS were measured every day for 5 days and the changes are represented in the Figures 3.

Different concentrations of the enzymes dose (50% of cellulase and 50% of amylase) such as 40U, 60U, 80U, 100U, 120U, and 140U are used to optimize the enzymatic hydrolysis. Based on Figure 4, 7, VS was initially around 115 g/L before digestion for each flasks containing different dose of enzymes and after the digestion at day 5, it decreased to 73.56 g/L, 71.31 g/L, 59.85 g/L, 72.24 g/L, 87.47 g/L and 101.23 g/L for 40U, 60U, 80U, 100U, 120U, and 140U of enzymes does respectively. Therefore, Deepanraj, *et al.* [33] have studied the influence of VS concentration of food waste on biogas production in anaerobic batch digester by considering different TS concentrations of food waste. The characteristics food waste used were determined before and after digestion. For the substrate with TS of 12.5 % before digestion, VS have decreased to 60.47 g/L respectively. Food waste with VS concentration of 100 g/L have reduced to 71.31 g/L whereas 80U/mL enzyme does was used. As a result, VS of the food waste have decreased due to the effect of the enzyme and this express the facilitation of the degradation of the organic compound based on the decrease of VS. Concerning the optimum does of enzymes, it is observed that enzymes does have no effect on the organic compound as the different does of enzymes gave VS values close to each other. This means that even small doses of enzymes can help organic compound to be degraded and improve hydrolysis of the food waste on the other hand, a control with 0 U/mL of enzyme which means food waste with no attached enzyme was also studied to see the effect of enzyme on food waste hydrolysis.

Figure-3. Effect in VS of food waste hydrolysis with different does of hydrolytic enzyme. Other factors were fixed pH 5, incubation time 5days, TS 12.5%, and room temperature 30°C (±2)



From the values of TS of the control it is obvious that there is no decreased and there was no degradation of organic compounds in hydrolysis of FW. Confer and Logan [34], found that microbe hydrolysis degrades dynamic insoluble material biomolecules such as polysaccharide and protein, follow by the discharge of hydrolytic protein molecules back into feed liquid. The enzyme dose was an influence on the degradation of the substrate [35].

3.2.4. Optimization of the Hydrolysis Process FCCCD under RSM

From the OFAT studies, the significant factors were imperilled to an optimization process in the form of FCCCD under RSM method to obtain the optimum enzymatic hydrolysis conditions for VS removal. During the design, interaction between factors can be studied. The key factor in optimization process is to improve and assess the statistical approach to gain a better understanding of the relationship between the factors intricate in the VS removal and to reduce the number and cost of experiments.

In the FCCCD, two factors were investigated, consisting of TS (10 to 15% g/L) enzyme does (60 to 100 U) and pH range (4.5 to 5.5) whereas other factors with less deviation toward reduce VS was fixed at OFAT optimum concentrations, as stated previously. Table 2 shows the actual and predicted values of the reducing sugar for each

experimental run, taken from the regression equation of 20 runs. Based on the results, the VS removal was 59 g/L at the center point of the design, and the lowest VS removal was observed in run 20. The regression coefficients of the equation were calculated using various regression analyses of the experimental data, and the fitted equation was utilized to forecast VS removal. The quadratic polynomial equation gave phases of VS elimination as a function of TS and enzyme activity, which may be written in terms of code factors, as indicated in the equation below. The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels are coded as -1. The coded equation (1) was useful for identifying the relative impact of the factors by comparing the factor coefficients.

VS= 60.13 - 0.364A - 0.56B - 6.38C - 0.102AB + 0.3AC + 2.05BC + 3.34A2 + 3.34B2 + 8.84C2 (1)

Where, VS is the (g/L) as a function of the coded levels of %TS (A) and enzyme does (B) and pH (C) respectively.

Table-2. FCCCD experimental design for selection of medium components and process conditions for VS removal

	Factor 1	**Factor 2**	**Factor 1**	**Response 2**	
Run	**A: TS**	**B: Enzyme Does**	**pH**	**VS (Actual)**	**VS (Predicted)**
	%	U/mL		g/L	g/L
1	12.5	80	4.5	75	75.35
2	**12.5**	**80**	**5**	**60.51**	**60.13**
3	10	100	4.5	80	80.19
4	15	60	5.5	68	67.83
5	**12.5**	**80**	**5**	**60.41**	**60.13**
6	10	100	5.5	70	70.93
7	10	80	5	65	63.83
8	**12.5**	**80**	**5**	**60**	**60.13**
9	**12.5**	**80**	**5**	**60.01**	**60.13**
10	12.5	80	5.5	63	62.59
11	10	60	5.5	67.91	67.75
12	**12.5**	**80**	**5**	**60.72**	**60.13**
13	15	100	5.5	70.79	70.60
14	12.5	60	5	63	64.03
15	15	100	4.5	78.48	78.65
16	**12.5**	**80**	**5**	**59**	**60.13**
17	10	60	4.5	85	85.21
18	12.5	100	5	64	62.91
19	15	60	4.5	85	84.09
20	15	80	5	62	63.11

***Bold indicates center points

Analysis of variance (ANOVA) of the response surface, the quadratic polynomial model for Response 1: VS was shown in Table 3. The F value of 170.77 of VS and p-value of <0.0001 of VS model indicates that the selected quadratic model was significant. P-value was also employed to explain the significance of each coefficient and utilized to observe the interaction strength between each coefficient that is independent.

The lower the p -value, the coefficient becomes more significant. P-value of <0.0001 implies that model terms are significant, whereas values greater than 0.1 means insignificant model terms. In this case the terms A, B, AB, A^2 and B^2 were found to be significant model and influence the overall reducing sugar production remarkable. Meanwhile, based on the F-values of the main factors studied, the substrate concentration presented the highest value, denoting that it presents the strongest influence on the VS removal, whereas the enzyme dose showed the least pronounced effect. The lack of fit F-value of 3.75 (VS) was also implies that the lack of fit is not significant relative to the pure error. Nonsignificant lack of fit indicated that the model fit adequately. The coefficient of determination (R^2) near to one which ensures a better correlation between the actual and predicted values. Furthermore, the efficiency of the model was displayed by the high value of R^2 = 0.9935, adjusted R^2 = 0.9978, predicted R^2= 0.9517 and adequate precision= 37.5673 of VS response. The signal to noise ratio was evaluated by the adequate precision, in which a ratio greater than 4 is considered a good model and the model studied demonstrated a ratio of 37.5673 of VS.

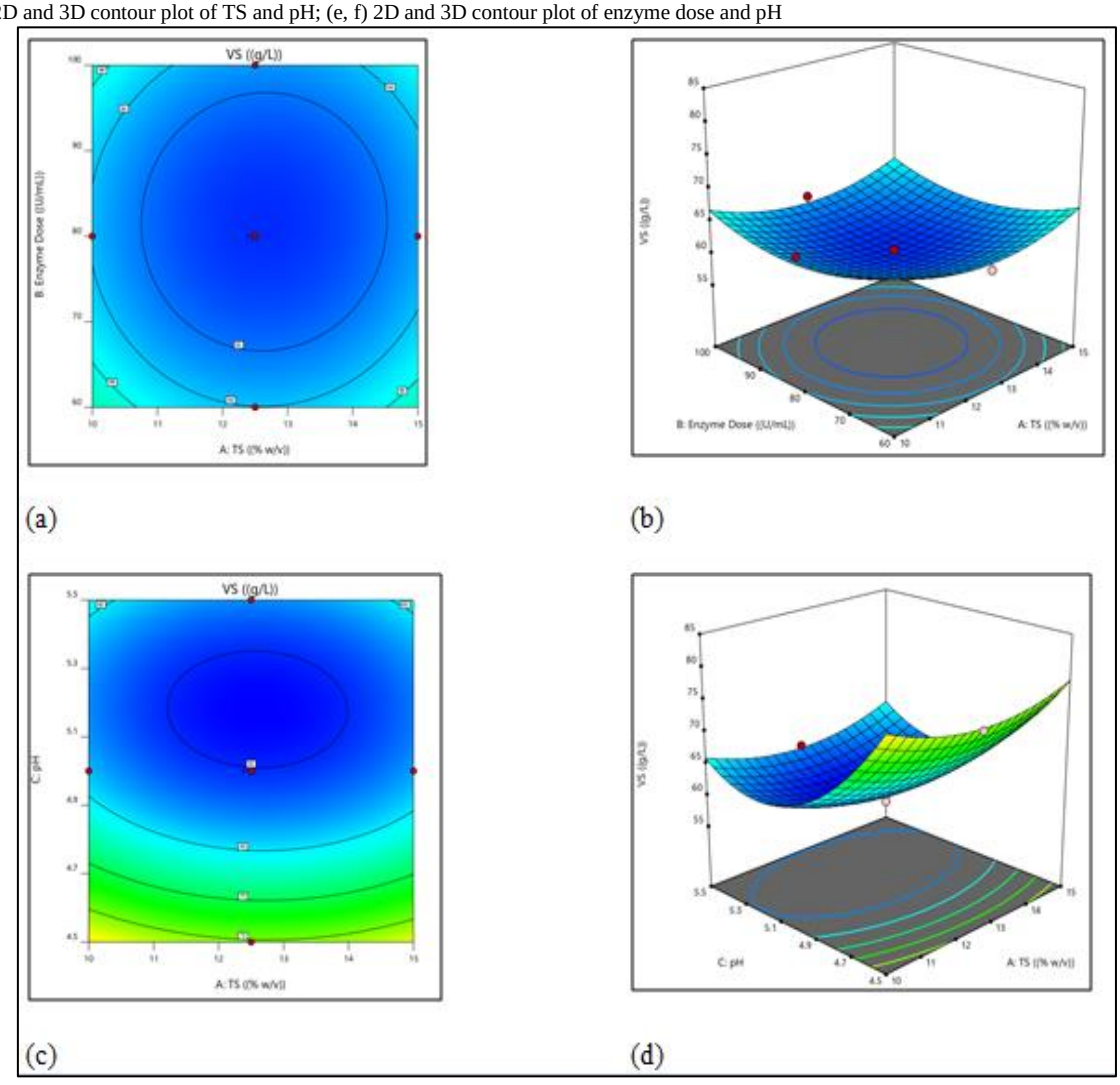
Table-3. Analysis of variance of the polynomial model for Response 1: VS

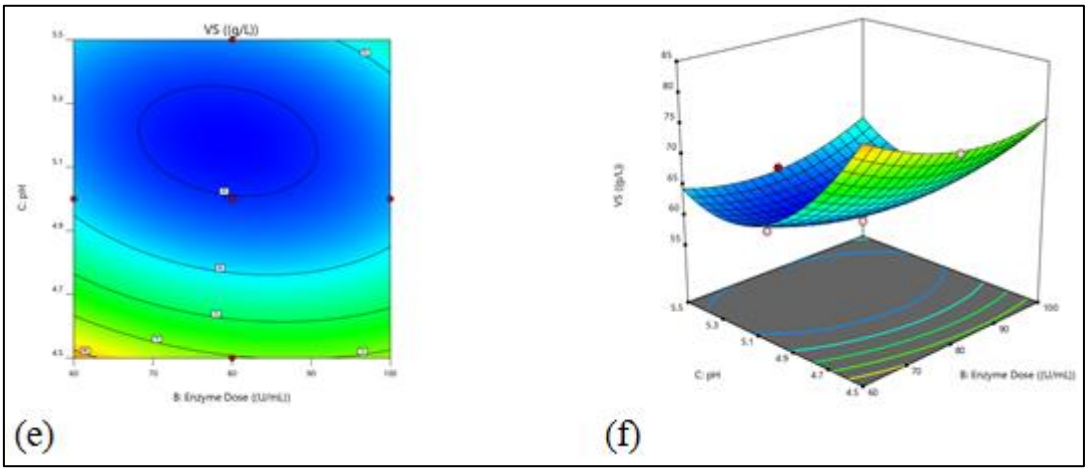
Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	1370.02	9	152.22	170.77	< 0.0001	significant
A-TS	1.32	1	1.32	1.49	0.2508	
B-Enzyme Dose	3.18	1	3.18	3.57	0.0882	
C-pH	406.79	1	406.79	456.34	< 0.0001	
AB	0.0840	1	0.0840	0.0943	0.7651	
AC	0.7200	1	0.7200	0.8077	0.3899	
BC	33.62	1	33.62	37.72	0.0001	
A^2	30.72	1	30.72	34.46	0.0002	
B^2	30.72	1	30.72	34.46	0.0002	
C^2	215.01	1	215.01	241.20	< 0.0001	
Residual	8.91	10	0.8914			
Lack of Fit	7.04	5	1.41	3.75	0.0866	not significant
Pure Error	1.88	5	0.3753			
Cor Total	1378.93	19				
R^2 = 0.993, Adjusted R^2 = 0.987, C.V.= 1.39, Predicted R^2= 0.951, Adequate precision= 37.56						

In the meantime, coefficient of variation (C.V.) defines the degree to which the data were distributed. The C.V. for VS removal was 1.39% which was within the acceptable range as small values of C.V. (Close to zero) give better reproducibility. A high C.V. implies high variation in the mean value and does not generate a satisfactory response model [36, 37].

The regression equation was employed to construct the contour (two-dimensional) and response surface (three-dimensional) plots utilized to examine the interaction between TS enzyme dose and pH to determine the optimum concentration of each factor for maximum VS removal. The plots show that VS removal was increased by the increment of TS enzyme dose and pH.

Figure-4. Interaction of food waste (TS), enzyme dose and pH on hydrolysis (g/L of VS): (a, b) 2D and 3D contour plot of TS and enzyme dose; (c, d) 2D and 3D contour plot of TS and pH; (e, f) 2D and 3D contour plot of enzyme dose and pH





The degree of the interactions between the variables is represented by the shape of the contour plots [38]. The three-dimensional (3D) response surface and two-dimensional (2D) contour plot of the interaction between TS (food waste), enzyme dose and pH were presented in Figure 4. It was discovered that decreasing the enzyme dosage enhanced the VS reduction, but increasing the enzyme dose suppressed the yield, which might be attributed to the saturation effect [38]. The maximum reduces of VS removal, 59g/L of food waste (Figure 4, 8), were obtained with the TS, an enzyme dose and pH of 12.5% (g/L) 80U/mL and 5, respectively.

3.2.5. Validation of the Model Developed: Hydrolysis of FW

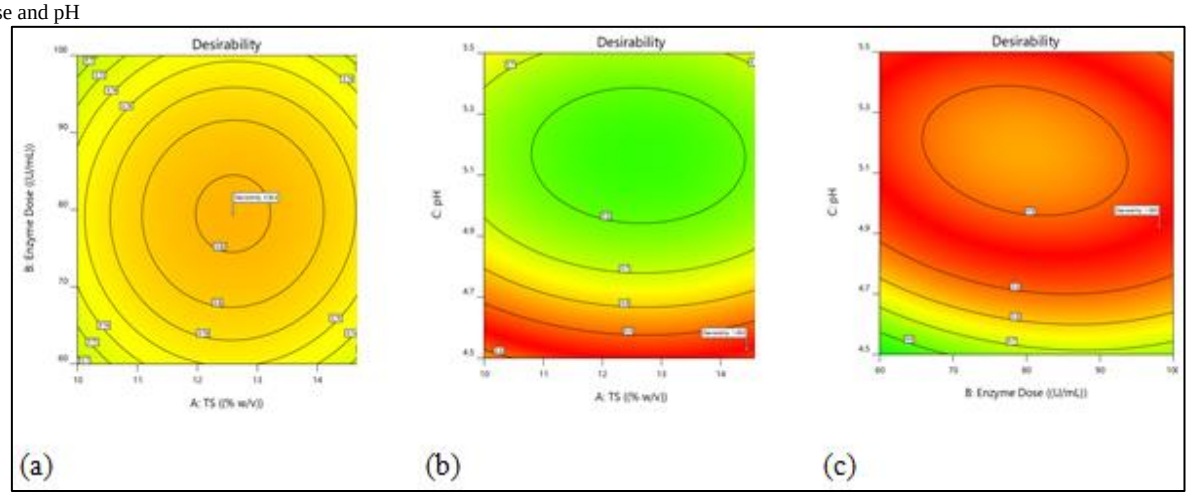
The statistical model was used to validate the proposed model by verifying the optimal findings. The created model was used to calculate various combinations of expected parameter values. Table 4 shows the process condition and combination for the hydrolysis of food waste constituted of elements of independent variables. The anticipated and experimental process conditions for the hydrolysis of food waste were determined to be within 1% of VS.

Table-4. Validation of the developed model enzymatic hydrolysis of food waste

Experiment	TS	Enzyme Dose	pH	VS (g/L)		
Number	(%)	(U)		Predicted Value	Experiment Value	Error (%)
1	12.59	79.47	5.19	58.97	59.45	0.80
2	14.44	85.37	4.53	75	76.03	1.35
3	14.5	98.08	4.92	65	65.85	1.29

As a result, it can be inferred that the proposed model is capable of predicting the reduction of FW hydrolysis. From this study of the developed enzymatic hydrolysis process, it was evident that the maximum removal 59.45 g/L of VS whereas TS (12.59 %), enzyme dose (79.47U/mL) and pH (5.19) were achieved due to the reduction of lignin and hemicellulose layer of the food waste during the pretreatment and the interaction of the other important combination, such as % TS enzyme dose and pH as shown in Figure 5.

Figure-5. 2D contour plots of predicted value three replications for desirability using (a) TS and enzyme does, (b) TS and pH and (c) enzyme dose and pH



Enzymatic hydrolysis of different lignocellulosic biomasses, like rice hulls [39], waste paper [40], food waste [41], sunflower stalks [42], water hyacinth [38], rice straw [43], etc., were studied. Among them, 30.3% removed was recorded by Ma, *et al.* [43] during saccharifying of rice straw, 57.8 % hydrolysis was removed from sunflower stalks [42] and 32% removed was achieved from rice hulls [39].

3.3. Study of Anaerobic Digestion Process of Food Waste

3.3.1. Characteristic of Anaerobic Inoculum

Different inoculums were previously used for anaerobic digestion of food waste and studies have evaluated the effect of different inoculum sources on the anaerobic digestion. It was noticed that it is important to know the characteristics of the inoculum used for anaerobic digestion to see the effects of the inoculum on the end product. As discussed, anaerobic sludge collected from local Dasherkandi Sewerage Treatment Plant was used as an inoculum in this study and some analyses were done in order to characterize the inoculum used. The following analysis was done according to the analytical methods described in the methodology chapter TS, VS and pH, as summarized in the Table 5. The inoculum used has a total solid content around 100 g/L and total volatile solid nearby 90 g/L.

Table-5. Characterization of inoculum

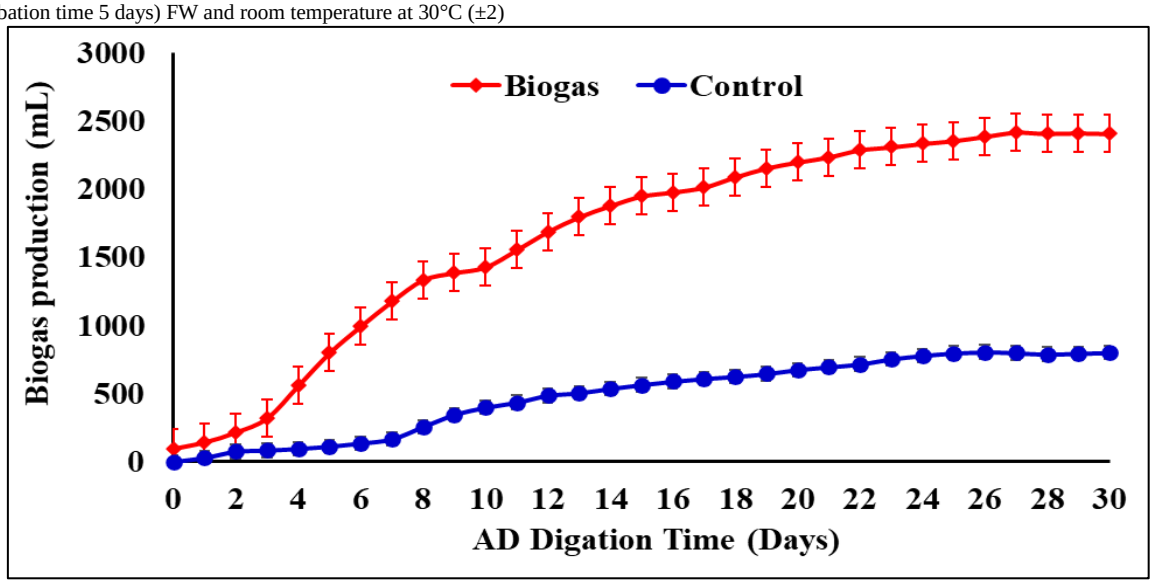
Parameters	Values
TS (g/L)	99.58
VS (g/L)	85.54
pH	5.21

3.3.2. One-Factor-At-A-Time (OFAT) Experimental Analysis for AD System

3.3.2.1. Digestion Time on AD System for Biogas Production

The experiment was to adopt a better AD retention time for the AD system of the considered food waste and also to exploit these results when using pilot scale in order to recover the maximum of energy from food waste. However, the AD system with different AD retention time were operated such as 0 and 30 days and biogas (volume of ppm using Arduino Uno based biogas sensor) produced was checked every day for 30 days and the evolutions of daily biogas and cumulative biogas for each AD retention time are represented in Figures 6. Based on Figure represented that the gas production was slow from the beginning until day 5 and it was pick up from 4 days to 9 days which was biogas volume was 560mL to 1387mL after that sharply increase of 27 days. Days 9 to days 15 were also rapidly increased which was around 1950.31mL in case other factors were fixed the biogas inoculum of 25%, pH of 7 and 500mL of hydrolysate (incubation time 5 days) FW and room temperature at 30°C (±2). In addition, days 15 to days 20 were little bit changed which was almost 2199.54mL. Biogas production was high for AD retention times were days 25 to 27 days but the AD retention time of 27 days was also able to produce significant amount of biogas which was 2418.62 mL.

Figure-6. Biogas production for different AD digestion times. Other factors were biogas inoculum of 25%, pH of 7, 500mL of hydrolysate (incubation time 5 days) FW and room temperature at 30°C (±2)

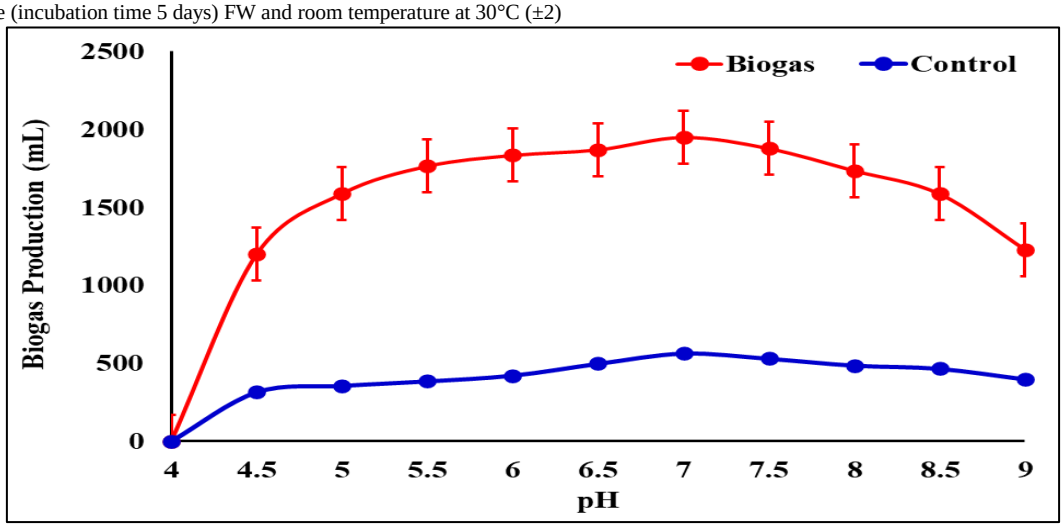


These results have confirmed good processing of methanization process and the importance of hydrolytic enzyme on biogas volume. As noticed by the researchers that in general, mesophilic condition can reach high production of biogas within 20 days to 30 days of AD retention time. The days 20 to days 25, it was clearly seen that the production of the biogas was reached almost 2356.45mL which is increased only 100ppm after that fall down. Haryanto, *et al.* [44] have operated anaerobic digestion with different AD retention time, namely 7, 14, 21, 28 and 35 days, with substrate concentration of 16 % (g/L) of (TS), and at the mesophilic temperature of 37°C. They have noticed that the biogas volume of AD retention time 14 day was quite similar to biogas volume of AD retention times 21, 28 and 35 days. Kim, *et al.* [45], studied the effects of temperature and hydraulic retention duration on AD with food waste as feed. They observed that AD capacity and food waste digestion efficiency improved at 50°C with a hydraulic retention duration of 12 days.

3.3.2.2. pH on AD system for Biogas Production

pH is a very significant parameter that has effect on anaerobic digestion process. The microbial inhabitants in the AD are very complex and it is essential to control pH in AD by a strong base such as NaoH or HCl. The aim of controlling pH was to evaluate initially in hydrolysis which has low pH values and avoid the accumulation of volatile fatty acids that can affect microbial inhabitants. However, in the AD system the range were of pH 6 to pH 8 whereas the optimum range was at pH 7 according to many researches [28, 46, 47]. Biogas was yield when the pH was at 7 as shown in the Figure 7. From Figure 7, pH was varied even though the pH was needed to adjust every day because the presence of microbes was not survived and decreased gas production in the AD. Initially the pH was acid (pH of 4.5) and increased slowly till pH of 9. From pH 4.5 to pH 6 were gradually increased biogas yield which were around 1200.25 mL to 1835.34 mL with 10 % of inoculum, AD digestion times of 27 days, 500mL of hydrolysate (incubation time 5 days) FW and room temperature at 30°C (±2). In contrast, pH 6 to pH 6.5 were slightly increased because the pH influences the chemical stabilities of NH_3, H_2S and volatile fatty acids, which could inhibit the bacteria activity. On the other hand, the pH 7 was maximum yield approximately of 1950.31mL biogas. The results illustrate that, biogas volume and degradation efficiency were substantially higher for pH ranged between 6 to7.5. From Figure 7, it was clearly seen that the pH 8 to pH 9 the production was fall down when pH was increased.

Figure-7. Biogas production for different initial pH. Other factors were biogas inoculum of 25%, AD digestion times of 15 days, 500mL of hydrolysate (incubation time 5 days) FW and room temperature at 30°C (±2)

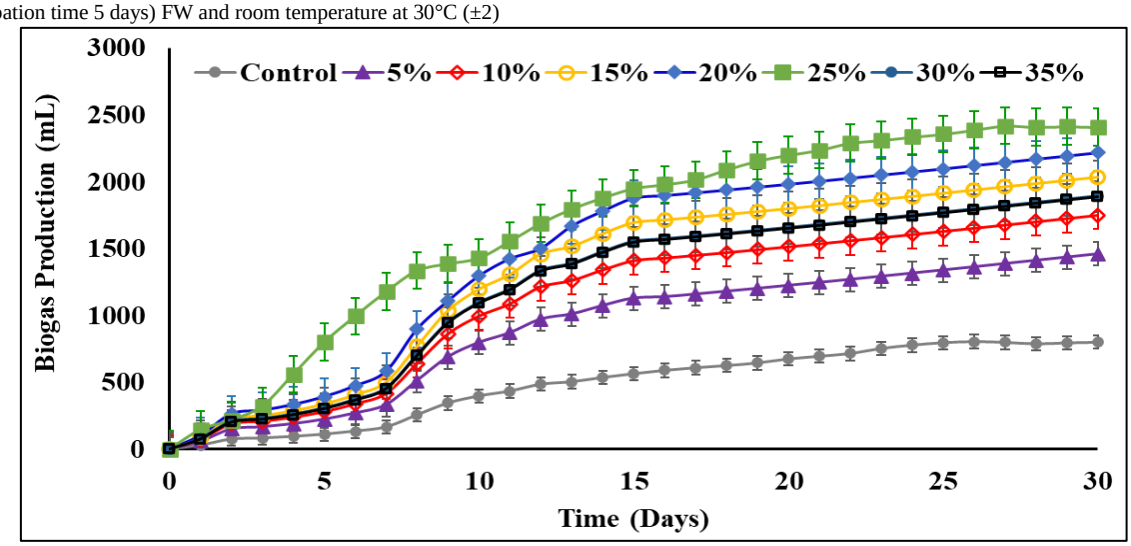


Consequently, the single AD with hydrolysed food waste presented an increase volume of biogas compared to Pavi, *et al.* [48] which found 493.8 mL/g VS of cumulated biogas was used pH 7 from fruit and vegetable waste. Additionally, Yang, *et al.* [49] found only 171.0 mL/g TS of biogas (pH 7) cumulative produced from food wastes collected from a canteen food waste, which consisted of rice, meats, vegetables, bones, etc.

3.3.2.3. Inoculum does on AD system for Biogas Production

The evolution of the biogas produced from hydrolysate (incubation time 5 days) FW with hydrolytic enzyme was evaluated. Daily productions of biogas found from the considered digesters with different percentage of inoculum dose and increasing of biogas produced are demonstrated in Figures 8. Firstly, daily biogas production was measured every day and recoded in AD retention time at 27 days. From Figures 8, the biogas production for the different percentage of inoculum dose was seen on the first day and continued growing slowly between days 3 to days 7 because the food waste used as a feedstock was a solid substrate which contained high carbohydrates that can slow down the start-up of the AD. Biogas production for different AD inoculum dose such as 5%, 10% 15%, 20%, 25% 30% and 35 % of inoculum were used to biogas production whereas other factors were fixed the AD digestion times of 27 days, 500mL of hydrolysate (incubation time 5 days) FW and room temperature at 30°C (±2). Inoculum dose 5%, 10% 15%, 20%, 25% 30% and 35 % were almost similar from days 1 to days 7 because broken down by microorganisms in the absence of oxygen. Days 8 to days 15 were gradually increased because food waste substrate were almost broken down to produce more sugar and microorganisms convert bioenergy like biogas and other gases. However, the AD 25% inoculum the production was maximum at days of 29 almost 2413.65mL. Concerning the daily biogas production of 25% of inoculum was speedily increased from days 3 to days 10 which were 322.54 and 1429.58mL respectively. Regarding the yield of the biogas, 5%, 10%, 15 %, and 20% were reached a high yield that makes a maximum of 60 to 500 mL at day 7 when the inoculum was used 20%.

Figure-8. Biogas production for different AD inoculum dose. Other factors were pH of 7, AD digestion times of 15 days, 500mL of hydrolysate (incubation time 5 days) FW and room temperature at 30°C (±2)

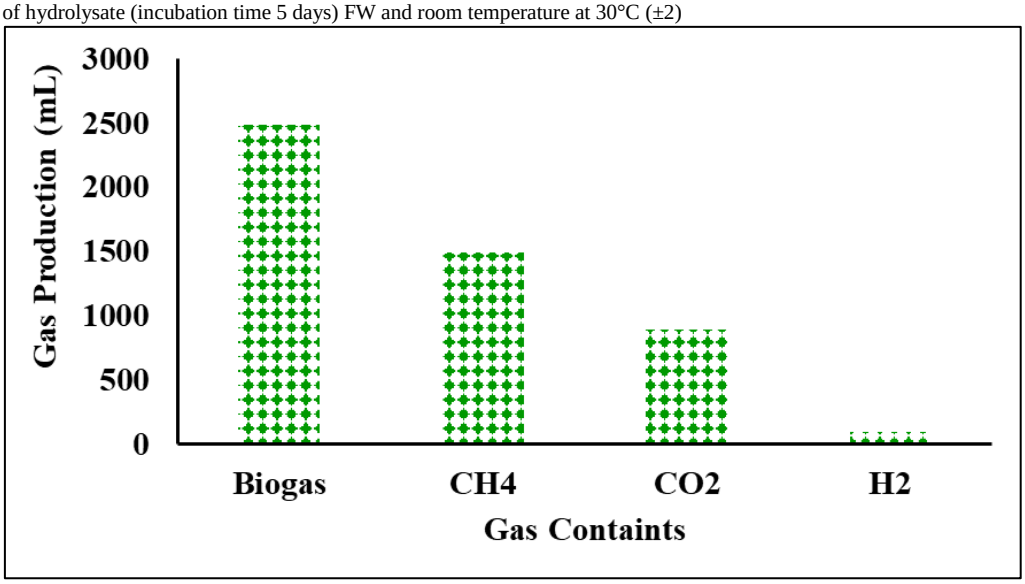


According to the initial condition represents 5% of inoculum was the lowest biogas volume and the slowest start up time. Also, the characterize of 5% inoculum and hydrolyzed food waste produced the lowest volume after the control comparing to the other ratios. The 15% of inoculum and containing food waste hydrolyzed was produced high volume of gas compared to the 5% of inoculum and the control. Lastly, the digester with high ratio of inoculum (25%) was the largest volume of biogas produced as shown in Figures 8. From the differences of the biogas produce from the control, it can be well argued that the hydrolysis has a huge effect on the anaerobic digestion of the food waste by helping the feedstock in the substantial production of biogas. However, the differences of the volume of biogas produced from the different inoculum ratios indicated the effect of the inoculum ratio on the biogas production. In other words, the volume of biogas produced has increased with the inoculum ratios [50]. In terms of inoculum ration, Lopes, *et al.* [51] investigated the role of digestion liquid inoculated in the breakdown of organic waste and discovered that the amount of indigenous anaerobic microbes in the digestion influences solid waste efficiency. Furthermore, Neves, *et al.* [52] discovered that employing particulate sludge as a spore suspension increased the yields in terms of CH_4 yields, indicating that there is an ideal feed to inoculation ratio that maximized CH_4 yields.

3.3.2.4. Biogas Production using Optimum Parameters

The rate of biogas oscillated, which might be attributable to the existence of a methylotroph species in the activated sludge, which utilizes CH_4 as a carbon and energy source for development. During the research, the total biogas created in the system was the sum of both carbon dioxide, hydrogen and methane. Biogas from the breakdown of food waste included %3 hydrogen, 57% methane and 40% carbon dioxide as shown in Figure 9. Biogas production was maximum using optimum parameters were fixed factors such as the biogas inoculum of 25%, pH of 7, AD digestion times of 29 days, 500mL of hydrolysate (incubation time 5 days) FW and room temperature at 30°C (±2). Using these parameters, the total biogas was approximately of 2490 mL which were includes 74.7mL of hydrogen, 1419.9mL of methane and 996mL of carbon dioxide.

Figure-9. Biogas production for optimum AD parameters. The fixed factors were biogas inoculum of 25%, pH of 7, AD digestion times of 29 days, 500mL of hydrolysate (incubation time 5 days) FW and room temperature at 30°C (±2)



Mohan and Jagadeesan [53] discovered that the breakdown of FW generated 76% CH_4 and 24% CO_2 in terms of biogas production percentage. Furthermore, the biogas comprises around 55% CH_4 and 30% CO_2 [54]. The determined exact biogas production found from this study (750.24 ± 34.0 mL/gVS added) at the substrate: inoculum of 40-80% was utilized than that of the determined production testified in the earlier studies showed with 554.0 ± 75.0 mL CH_4/gVS added [55] and AD of FW, 242.69 mL CH_4/gVS added [56].

4. Conclusion

Biogas production from food waste using enzymatic hydrolysis was a promising technology that involves breaking down organic material in food waste to produce biogas, a mixture of methane and carbon dioxide. Enzymatic hydrolysis was a process that involves using enzymes to break down the complex organic molecules in food waste into simpler molecules that can be easily digested by bacteria to produce biogas. Therefore, it was significant to find an amount of hydrolytic enzymes dose and digestion time optimum for hydrolysis of food waste. For that, the effect of hydrolytic enzymes amount and digestion time were examined by one factor at-a-time process with respect to TS, VS, and COD changes. In this study, as a source of high concentration of food waste were used to produce various methogenesis compounds using cellulase and amylase enzyme by means of OFAT method and FCCCD. However, the classical OFAT studies revealed that hydrolysis pH, hydrolysis time and enzyme dose were associated with degrading lignocellulose biomass and produce different biodegrade organic compounds. Based on this, a second-order regression model was developed by optimizing these two components using FCCCD. The reduction of sugar output was successfully improved. As observed from the results, any number of hydrolytic enzymes is susceptible to biodegrade organic matter and increase hydrolysis of food waste even though the optimal digestion time was found to be long (5 days). This can be explained by the fact that food waste composed of big polymers that need time to be degraded. Overall finding of the current study has revealed that the addition of hydrolytic enzymes in the hydrolysis reactor can enhance the degradation of the organic matter of food waste.

Biogas yield was optimum using OFAT parameters such as the biogas inoculum of 25%, pH of 7, AD digestion times of 29 days, 500mL of hydrolysate (incubation time 5 days) FW and room temperature at 30°C (±2). The optimum OFAT parameters which give high production of biogas was determined to be the AD systems. The optimum value for inoculum to feed ratio was 25 %, while the optimum AD digestion time was staring from 29 days. As a result, the application on food waste hydrolysis showed a promising result where 11.15 % of volatile solid was reduced almost 50% and 5.58 % of VS was removed with only 29 days of digestion. However, the AD system was studied and the result showed that biogas contain was found such as %3 hydrogen, 70% methane and 27% carbon dioxide. The benefits of biogas production from food waste using enzymatic hydrolysis include reducing greenhouse gas emissions by diverting food waste from landfills and producing a renewable energy source. Additionally, the process can help to reduce the amount of waste generated in landfills, which can be a source of pollution and environmental degradation.

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