Advances in Environmental Research, *Vol. 9, No. 1 (2020) 55-64*
DOI: https://doi.org/10.12989/aer.2020.9.1.055

Digestate residues analysis under elevated heat regime by using DNS method

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(Received June 15, 2019, Revised March 17, 2020, Accepted March 27, 2020)

Abstract. The problems with unsorted municipal waste are always associated with disposal issues as it requires a large area for landfilling or high energy used for incineration. In recent years, an autoclaving technique has been considered a promising approach which could minimize the volume of organic waste from being directly disposed or incinerated. In this work, an attempt was done to study the saccharification potential of organic residues under elevated temperature Thermal treatment involving hot water bath was applied to treat the organic residue ranging from 60℃ to 100℃ for 30 and 60 minutes. The result obtained showed an increasing trend for the concentration of glucose and carbohydrate. However, the result for lignocellulose content which contains various component includes extractive, holocellulose, hemicellulose, cellulose and lignin show variation. Based on the thermal treatment carried out, the result indicated that the trend of glucose and carbohydrate content. The highest percentage of glucose that can be obtained 978.602 µg/ml which could be obtained at 90℃ at 60 minutes. The carbohydrate also shows an increasing trend with 0.234 mg/ml as the highest peak achieved at 80℃ for 30 minutes treatment. However, it was found that the lignocellulose content varies with temperature and time. The statistical analysis was carried out using two-ways ANOVA shows an interaction effect between the independent variables (temperature and contact time) and the saccharification effects on the food wastes. The result shows a variation in the significant effect of independent variables on the changes in the composition of food waste.

Keywords: cellulose; DNS; heat treatment; hemicellulose; holocellulose; lignin; lignocellulose; organic residue

1. Introduction

The management of solid waste remains a major issue worldwide. The environmental quality is deteriorating due to improper solid waste disposal and becoming a huge challenge in the

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developing country to determine an appropriate way of managing the waste(Kamaruddin *et al.*, 2019). Malaysia is not excluded from facing the same problem. The land scarcity has caused the implementation of solid waste management involving the landfilling process is considered unsuitable in meeting ever-increasing generation of municipal solid waste (MSW). Disposal of solid waste could be managed by any means of destruction, incineration, and deposit or decomposing according to the Malaysia Solid Waste and Public Cleansing Management Act 2007 (Act 672).

The changes in the generation rates of MSW are commonly caused by several factors include demographics, facilities, nature, level of economics, urbanization level, cultural norms, energy source, weather conditions and lifestyle of Malaysian population (Abd Manaf *et al.* 2009). Based on the data recorded National Solid Waste Management Department of Malaysia, the waste generated shows different weight fractions between six regions in Malaysia. The data shows that the region with higher population density and economic development generated the highest amount of waste. Typical characteristics of MSW in Malaysia contains a very high amount of organic waste with high moisture content and bulk density approximately above 200 kg/m^3 (Abd Manaf *et al.* 2009).

The highest composition of waste that can be found in the waste fraction is food waste with almost 45% of the total solid waste generated. The high potential of food waste for treatment purposes have been implemented through various kinds of treatment methods such as composting, incinerating, autoclaving. By exploiting the waste through this approach, it could reduce disposal costs transportation costs that able to prolong the life spans of landfill sites. The food waste which derived from plant-based material contains a high amount of lignocellulose which is valuable to be retrieved. The abundance and the renewability of lignocellulose material made from food waste ideal for recovery and utilization for the value-added product (Foyle *et al.* 2007). The utilization of organics residue from food waste could be a potential source for lignin. In fact, proteins and sugars from food waste could also be extracted although the lipids and cellulose are little degradable, while the keratins could be slowly degraded.

Pre-treatment is normally carried out to increase the degradation rate and improve feedstock availability (Kumar *et al.* 2009). Various pre-treatment is available for a different type of feedstock (Wordofa 2014). Pre-treatment is divided into several techniques include physical, chemical, biological pre-treatment. In this study, food waste was selected to be treated by using a hot water bath which was thermal pre-treatment. Thermal treatment involves the process of heating lignocellulosic material to a certain temperature to destruct the structure of materials.

There are various thermal treatment methods such as liquid hot water, steam, autohydrolysis and aquasolv treatment (Triantafyllidis *et al.* 2013). In this study, thermal treatment involves the addition of food waste in a small-scale flask to be treated in a hot water bath. The mode of heat transfer in hot water bath initially involves convection followed by conduction (Barua and Kalamdhad 2017). The glassware base had direct contact with the hot water that enabled the sample to undergo treatment. The hot water is set to a specific temperature and specific time to allow heat to pass to the lignocellulosic sample. The formation of an inhibitor such as phenolic compound is the main limitation for this treatment process. The high-water input in liquid hot water treatment will inhibit the formation of inhibitor (Sims 2013). The pre-treatment enables to reduce the amount of lignin content in the food waste. There are several factors affecting the thermal treatment products. Natural factors such as high moisture content and particle size while physical factors such as temperature and contact time contribute a significant effect on the thermal treatment process (Karthikeyan *et al.* 2018). The purpose of this work is to determine the effect of

thermal treatment by hydrolysis on the characteristic of food waste such as the content of glucose, carbohydrate and lignocellulose. This work also aims to determine the relationship between the time of contact and temperature towards the content of glucose, carbohydrate and lignocellulose.

2. Materials and methods

2.1 Sample collection

The food waste used in the experiment was collected from a restaurant in Penang, Malaysia (5°21'24.3"N 100°18'15.7"E). The strategic location of the restaurant contributed to a large amount of food waste generated. The food waste was collected and separated into organic and inorganic waste. Only organic waste was selected and further processed. Food waste contains high moisture content that needs to be removed before any treatment being done to the waste. Food waste was spread on a tray and retained in Memmert Oven for 60^{o}C for one day as shown in Fig. 1. This would allow the moisture content to be reduced. After being dried, the food waste sample was blended using Heavy Duty Blender HGB550 to reduce the size of food waste and increase the surface areas**.** The sample was sealed in a plastic bag and stored in a freezer at a temperature of 4^{o}C.

2.2 Thermal pre-treatment

The food waste was placed into a conical flask of 250 ml volume. The ratio for food waste to the water was 1:10**.** 25g of food waste was inserted into the flask followed by the water and the flask was sealed upon completion of the process. The flask containing the food waste sample was heated at 105^{o}C, 70^{o}C, 80^{o}C, 90^{o}C, 100^{o}C for 30 and 60 minutes(del Campo *et al.*, 2006). Fig. 1 shows a sample of oven dried sample of the food waste.

2.3 Glucose determination

In this study, the method that was used to carry out the determination of glucose was Dinitrosalicyclic Acid (DNS) method. This method is easy to carry out, sensitive and adaptable

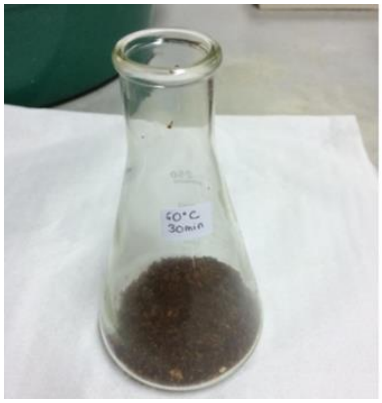

Fig. 1 Oven dried sample

during handling of a large number of samples at a time. The materials required consist of Dinitrosalicyclic Acid Reagent (DNS Reagent**)** and 40% Rochelle salt solution which is the potassium sodium tartarate. The DNS reagent was prepared by dissolving 1 g of dinitrosalicyclic acid with 200 mg crystalline phenol and 50 mg sodium sulfite in 100 mL 1% NaOH. The solution was then stored at 4^{o}C. The addition of sodium sulfite to the reagent for long a storage would cause the reagent to deteriorate. Sodium sulfite was added only during for the time of use. 100 mg of food waste sample was weighted and inserted into a flask. 5 mL of hot 80% ethanol was inserted into the flask to extract the sugar. The solution containing the sample was centrifuged and the supernatant was collected. The supernatant was evaporated by keeping it on a water bath at 80^{o}C. The process of extracting the sugar was repeated twice. 10 mL of water was added to dissolve the sugar. 2 mL of the extractive was pipetted out and inserted into the vial. 3 mL of DNS reagent was added into the vial. The vial was capped close and heated in a boiling water bath for 5 min. 1 mL of 40% Rochelle salt solution was added when the contents of the vial were still warm. The vial was let cool and the intensity of dark red color was read at 510 nm using a spectrophotometer (DR2010, Hach). A series of standard was run using glucose range from 0 until 5000 µg with 1000 µg interval. A standard graph was plotted and the amount of glucose present in the sample was determined using the standard graph.

2.4 Carbohydrate determination

The determination of carbohydrate in this study was carried out based on Anthrone Method (Ohemeng-Ntiamoah and Datta 2018). The general principle of this method is the hydrolyzing the carbohydrate contents into simple sugars by using dilute hydrochloric acid. 100 mg of the sample was weighed and inserted into the vial. 5 mL of 2.5 N HCL was added and keep it in a boiling water bath for three hours. The vial was let cool to room temperature. Sodium carbonate was added to neutralize the solution until the effervescence ceases. The solution was poured into a flask and the volume was makeup to 100 ml. The supernatant was collected and 1 mL of the aliquots were taken for analysis. 4 mL of anthrone reagent was added before heated for eight minutes in a boiling water bath. The vial was let cool and read the green to dark green color at 630 nm using spectrophotometer (DR2010, Hach). The standard glucose was prepared by dissolving 100 mg of glucose in 100 mL distilled water. 10 mL of stock was diluted to 100 mL with distilled water and serves as a working standard.

2.5 Lignocellulose determination

Lignocellulose consists of cellulose, hemicellulose, lignin and a small portion of extractive. The components of lignocellulose calculated in percent of oven dry weight. The determination of lignocellulose was following the TAPPI Standard Method.

2.5.1 Holo-cellulose determination

Holocellulose is the product of hemicellulose and cellulose. 3 g of food waste sample of free extractive was weighted and inserted into a 250 ml conical flask. 1.5 g of sodium chlorite ($NaClO_2$) was dissolved in 160 ml of distilled water. 10 drops of acetic acid added to the solution was mixed with the sample. The opening of the flask was capped with a small conical flask to allow reflux. Oxidation occurs at temperature 75^{o}C and carried out for 3 hours. The solution was added with 1.5 g $NaClO_2$ and 10 drops of acetic acid every 1hour interval. The flask was cooled a

filtered. The sample was then washed with cold water during the filtering process until neutral which can be determined by the diminishing of bubbles that formed. Acetone was added before the sample was further dried in the oven at 40^{o}C until a constant weight was obtained.

2.5.2 Cellulose determination

Cellulose is part of holocellulose and needs to be separated to obtain the pure content. 2 g of holocellulose was weighted and inserted into a small conical which immersed into a container containing ice to maintain the temperature below 20^{o}C for 30 minutes. 10 ml of 17.5% of NaOH solution was added into the flask and stirred before left for 20 minutes under a controlled temperature. After 20 minutes, 10 ml of distilled water was added and stirred. The solution then filtered until 16 ml of the filtrate obtained. The sample then washed with cold distilled water until 200 ml of the filtrate obtained and the filtrate was kept for hemicellulose determination in Section 3.3.3d. 8 ml of acetic acid was added to the filtering process of the sample before washed with 200 ml of hot water. The sample was dried in the oven at 105^{o}C until a constant weight obtained.

2.5.3 Hemicellulose determination

Hemicellulose is part of cellulose that easily dissolve. Nice medium filter papers with a size of 12.5 cm used in this experiment were dried in the oven at 105^{o}C, cooled in the desiccator and weighted. The filtrate that was kept from section 3.3.3a was poured into a flask. 16 ml of 30% acetic acid was added into the flask and placed in a water bath. The mixture was heated slowly until hemicellulose formed in the flask. The mixture was let cool before filtered using filter paper that already known the weight and washed with warm water. The filter paper that contains the hemicellulose was dried in the oven until a constant weight was obtained.

2.5.4 Lignin determination

1 g of free extractive food waste was weighted and inserted into a small flask of 50 ml. 25 ml of 72% sulphuric acid was added slowly while stirred with a glass rod. The flask then was cooled in an ice container to maintain the temperature below 20^{o}C for 2 hours. The mixture then poured into a flask of 1000 ml and 560 ml of distilled water was added into the flask. The opening of the flask was capped with the smaller flask to allow reflux. The flask then was heated until boil for 4 hours before being let cooled and the sample settles down of the flask. The solution was filtered and washed with 500 ml of hot water before dried in the oven until constant weight obtained.

3. Results and discussions

3.1 Glucose determination analysis

One of the factors contributing to fertilizer quality was the glucose content after thermally treated. Based on the laboratory work, the glucose content was measured by comparing the absorbance of the sample with the standard curve of glucose. The standard curve had a linear graph with an equation of $y = 0.001x - 0.023$ and the coefficient of determination (R^2) with a value of 0.9481 (Fig. not shown). The R square value which was near to 1 shows that the graph plotted was linearly projected and suitable to be used to determine the glucose content. The glucose content was obtained by matching the absorbance with the linear graph of the curve, matching with the concentration of standard of glucose. The rate of glucose content increases with the

Table 1 Standard deviation of each temperature and time reading during observations of glucose contents

Time/ Temperature (°C)	30 minutes	Percentage of different (%)	60 minutes	Percentage of different (%)
Raw	145.174 ± 42.117	0	145.174 ± 42.117	0
60	267.180 ± 45.936	84.04	369.009 ± 14.447	154.18
70	384.457 ± 44.148	164.83	466.425 ± 9.661	221.29
80	397.383 ± 57.696	173.73	469.577 ± 38.731	223.46
90	848.835 ± 7.386	484.70	978.602 ± 0.675	574.09
100	900.853 ±	520.53	974.309 ±	571.13

increase of the temperature. Cellulose consists of a long chain of sugar molecules while hemicellulose involves the shorter chain (Huang *et al.* 2015). The thermal treatment caused the long chain of cellulose to break down at any indefinite point into glucose fragment of different sizes (Barua and Kalamdhad 2017). The highest glucose concentration measured was at 90℃ at 60 minutes duration of thermal treatment with a concentration of 978.602 µg/ml which show an increase of 574.09%. However, the glucose content for 60 minutes treatment shows a sudden reduce at temperature 100°C. Table 1 summarizes the standard deviation and time reading for glucose contents.

3.2 Carbohydrate determination analysis

The carbohydrates concentration which known as polysaccharides also an important parameter to determine the quality of fertilizer produced. The concentration of carbohydrates determined through the sample absorbance result with the standard curve of carbohydrates. The standard curve has a linear equation of y = 11.516x - 0.026 and R square of 0.9792 (Fig. not shown). The concentration of carbohydrates shows an increase with the temperature. The concentration of carbohydrates with respect to absorbance exceeding the standard maximum level. Hence extrapolation was used to indicate the amount of carbohydrate concentration beyond the standard range (Jung *et al.* 2015). The highest concentration of carbohydrate recorded was at 80℃ and 30 minutes treatment with 254.55% increase. However, at 90°C, there is a sudden reduction to the concentration for both samples that thermally treated for 30 minutes and 60 minutes as simplified in Table 2.

3.3 Lignocellulose components analysis

Table 3 shows the variation of result for every component of lignocellulose in the treated food waste samples. Based on the result, there are variations in each component which affected due to several factors. The extractive composition which was expected to be low showed variation which causes a high and low extractive content extracted. The holocellulose showed a total of hemicellulose and cellulose. The hemicellulose was found to be decreased with the increase of time and temperature of treatment. The cellulose contents showed an increase with time and temperature. According to(Jönsson and Martín 2016), the operational conditions were tuned to allow the optimal digestibility of cellulose by removing the hemicellulose and/or lignin from the

Table 3 The composition of lignocellulose components

Composition (%) / Time (Min)		Extractive (%)	Holo-cellulose (%)	Hemi-cellulose (%)	Cellulose (%)	Lignin (%)
Raw	-	16.88	43.06	4.82	38.24	20.18
60°C	30 Min	4.18	48.26	20.23	27.94	20.6
	60 Min	17.89	43.36	12.33	31.03	7.0
70°C	30 Min	18.18	52.59	21.72	30.57	15.23
	60 Min	31.47	42.66	6.02	36.64	13.61
80°C	30 Min	21.52	45.14	9.41	35.73	20.08
	60 Min	26.36	45.6	7.25	38.35	16.44
90°C	30 Min	11.35	41.4	1.99	39.41	9.28
	60 Min	30.88	44.47	4.76	39.71	21.01
100°C	30 Min	25.6	61.36	4.93	39.41	7.28
	60 Min	17.9	57.2	14.79	42.41	11.83

Table 4 ANOVA data of temperature and time of glucose in sample

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	2166145.593[a]	9	240682.844	229.682	0.000	0.990
Intercept	11004836.140	1	11004836.140	10501.835	0.000	0.998
time	63263.341	1	63263.341	60.372	0.000	0.751
temperature	2099342.826	4	524835.707	500.847	0.000	0.990
time * temperature	3539.425	4	884.856	0.844	0.513	0.144
Error	20957.930	20	1047.897			
Total	13191939.670	30				
Corrected Total	2187103.523	29				

a. R Squared = 0.990 (Adjusted R Squared = 0.986)

matrix of lignocellulosic biomass. In order to obtain a high degree of solubilization hemicellulose and/or lignin, the degradation of solubilization fragment was unavoidable. Non-structural carbohydrates which include the cellulose and hemicellulose content could also be removed by extraction due to ethanol (Yu *et al.* 2011). This could be a possible reason contributing to the high amount of extractive obtained (Karimi and Taherzadeh 2016).

3.4 Statistical analysis

The statistical data analysis involves the determination of p-value and the correlation and regression analysis. The p-value shows the statistically significant interaction between the dependent and independent variables. The p-value lower than $p<0.05$ shows a significant interaction while the result above the value shows otherwise. The correlation and regression

Table 5 ANOVA data of temperature and time of carbohydrate in sample

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	0.056[a]	9	0.006	399.287	0.000	0.994
Intercept	0.874	1	0.874	56057.444	0.000	1.000
time	0.001	1	0.001	56.077	0.000	0.737
temperature	0.047	4	0.012	745.521	0.000	0.993
time * temperature	0.009	4	0.002	138.855	0.000	0.965
Error	0.000	20	1.560E-5			
Total	0.931	30				
Corrected Total	0.056	29				

a.R Squared = 0.994 (Adjusted R Squared = 0.992)

analysis show the strength and the positivity or negativity of the correlation and regression analysis. Table 4 shows the ANOVA data for temperature and time for glucose content in the sample. The data showed that there are statistically significant interactions for individual time and temperature to the glucose content. The significant value observed for the interaction of time and temperature is 0.513 (p = 0.513) which is more than 0.05. The interaction of time and temperature has no statistically significant difference in glucose content. The regression coefficient for temperature against the concentration glucose at 30 minutes is 0.936 which show positive strong regression. The regression coefficient for temperature against the concentration glucose at 60 minutes is 0.910 which show positive strong regression.

Table 5 shows the ANOVA data for temperature and time for carbohydrate content in the sample. The data showed that there are statistically significant interactions for individual time and temperature to the carbohydrate content. The interaction of time and temperature has a statistically significant difference to the carbohydrate content. The regression coefficient for temperature against the concentration carbohydrate at 30 minutes is 0.526 which show positive medium regression. The regression coefficient for temperature against the concentration carbohydrate at 60 minutes is 0.521 which show positive medium regression.

ANOVA for time and temperature to the lignocellulose content compromising the extractive, holocellulose, hemicellulose, cellulose and lignin are calculated. Every component of lignocellulose was analyzed with respect to contact time and temperature. The extractive shows no statistically significant to time and temperature. The correlation for extractive to the temperature and time showed that both have a positive correlation. The strength for correlation of extractive to temperature is weak while the strength for correlation extractive to time is medium strength. The holocellulose show no statistically significant with both time and temperature. The strength for correlation for holocellulose to time is negative weak correlation while the strength for holocellulose to temperature is medium positive correlation. The hemicellulose showed no statistically significant between hemicellulose with time and temperature. The correlation for hemicellulose to both time and temperature show a negative weak and medium correlation strength. The cellulose also shows no statistically significant interaction between cellulose with time and temperature. The correlation strength for cellulose towards the time is positive weak correlation while for cellulose towards the temperature was a positive strong correlation. The lignin shows no statistically significant interaction between lignin with temperature and time. The correlation strength is weak for both time and temperature towards the lignin.

4. Conclusions

The disposal of food waste reducing one of the important sources for the nutrient cycle. The improper management of food waste tends to cause the rise of problems to the final disposal of the solid waste which is the landfilling. The study was carried out to discover the options for treatment of food waste which can be utilized as value-added products including fertilizer production. Based on the study carried out, the food waste was treated using hot water bath technic which was one of the common pre-treatment methods that been used widely. The food waste was treated under five different temperature (60℃- 100℃) with two different contact time (30 minutes and 60 minutes). Based on the thermal treatment carried out, the result indicated that the trend of glucose and carbohydrate content. The highest percentage of glucose that can be obtained 978.602 µg/ml which can be obtained at 90℃ at 60 minutes. The carbohydrate also shows an increasing trend with 0.234 mg/ml as the highest peak achieved at 80℃ for 30 minutes treatment. The lignocellulose content varies with temperature and time. The composition differs for every treatment temperature and time. The statistical analysis was carried out using two-ways ANOVA shows an interaction effect between the independent variables (temperature and contact time) and the saccharification effects on the food wastes. The result shows a variation insignificant effect of independent variables on the changes of the composition of food waste. Further study on the use of thermal treatment is required to achieve faster yield saccharification and for a better understanding of the system to identify further improvements. One of the important components in this study which was the moisture content should be maintained throughout the study to ensure that the result obtained to verify the initial content of the sample. The thermal treatment shows an increase of saccharification with temperature and time. Further retention time and temperature can be considered to determine the effect on the yield of saccharification.

Acknowledgments

Authors gratefully acknowledge the financial support received under Research University Grant for Short Term Scheme from Universiti Sains Malaysia that enabled this work to be carried out (304/PTEKIND/6315062).

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