



# **ANAEROBIC DIGESTION OF BIOGAS PRODUCTION FROM THE COMMERCIAL FOOD WASTE SOURCE AND COW DUNG INOCULUM**

This report is submitted in accordance with requirement of the Universiti Teknikal Malaysia Melaka (UTeM) for Bachelor Degree of Manufacturing Engineering (Hons.)



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## DECLARATION

I hereby, declared this report entitled “Anaerobic Digestion of Biogas Production from the Commercial Food Waste Source and Cow Dung Inoculum” is the result of my own research except as cited in references.

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## **APPROVAL**

This report is submitted to the Faculty of Manufacturing Engineering of Universiti Teknikal Malaysia Melaka as a partial fulfilment of the requirement for Degree of Manufacturing Engineering (Hons). The member of the supervisory committee is as follow:



## ABSTRAK

Pencernaan anaerob adalah teknologi yang layak dengan potensi tertinggi untuk menukar sisa makanan menjadi tenaga yang boleh diperbaharui. Oleh itu, banyak penyelidik telah meneliti pendekatan pencernaan anaerob dalam kajian mereka untuk meningkatkan pengeluaran biogas. Selanjutnya, penyelidikan yang menggunakan jenis sumber sisa makanan tertentu untuk pengeluaran biogas, untuk menghasilkan jumlah hasil biohidrogen yang relevan masih belum mencukupi. Oleh itu, penyelidikan ini telah dijalankan untuk mengesan pengeluaran gas biohidrogen dengan memberi tumpuan kepada jenis sumber sisa makanan tertentu. Kemudian, komposisi kimia sumber sisa makanan terpilih dianalisis dengan menggunakan analisis Fourier transform infrared (FTIR). Melalui analisis FTIR, didapati bahawa sifat komposisi kimia untuk substrat yang digunakan untuk pengeluaran biohidrogen difahami sepenuhnya. Tujuan kajian ini adalah untuk mengesan gas biohidrogen dengan menggunakan alat analisis Gas Chromatography-Thermal Conductivity Detector (GC-TCD). Oleh itu, nisbah gas yang dikumpulkan dari ayam ke nisbah sisa beras (1: 1), (1: 2) dan (2: 1) dinilai melalui ujian analisis GC-TCD. Melalui ujian pengesanan gas, didapati bahawa nisbah substrat 1: 2 telah mendorong kemungkinan pengeluaran biohidrogen lebih tinggi. Hingga kini, sebahagian besar penyelidikan memfokuskan pada faktor pH, nisbah C / N dan suhu pada pengeluaran biohidrogen. Walaupun begitu, masih belum ada kajian atau kajian yang serupa mengenai nisbah substrat antara sisa ayam dan sisa beras. Oleh itu, korelasi nisbah substrat antara sisa ayam dan sisa beras yang telah menyebabkan pengeluaran biogas dan hasil gas biohidrogen lebih tinggi dinilai dalam penyelidikan ini. Pada akhir kajian ini, reaktor mini biogas disahkan berfungsi dan berpotensi menghasilkan gas biohidrogen yang berjaya dikesan dari ujian analisis gas GC-TCD. Penyelidikan ini sangat penting untuk dilakukan kerana memberikan alternatif lain untuk menghasilkan alternatif biohidrogen hijau dari sumber sisa makanan yang bermanfaat untuk persekitaran yang lestari dan hijau.

## ABSTRACT

Anaerobic digestion is feasible technology with highest potential to convert food waste into renewable energy. Therefore, many researchers have investigated anaerobic digestion approach in their studies for enhancing biogas production. Furthermore, research utilizing specific types of food waste sources for biogas production, to produce relevant amount of biohydrogen yield are still at scarce. Hence, this research has carried out to detect biohydrogen gas production by focusing on specific type of food waste source. Later, the chemical composition of selected food waste source was analysed by using Fourier transform infrared (FTIR) analysis. Through FTIR analysis, it was found that the nature of chemical composition for substrate used for biohydrogen production was fully understood. The purpose of this study is to detect biohydrogen gases by using a Gas Chromatography-Thermal Conductivity Detector (GC-TCD) analytical equipment. Therefore, the collected gases from chicken to rice waste ratios of (1:1), (1:2) and (2:1) were evaluated through GC-TCD analysis testing. Through gas detection testing, it was found that the substrate ratio of 1:2 had promoted higher possibility of biohydrogen production. Up until now, most of the research has mainly focused on the factors of pH, C/N ratio and temperature on biohydrogen production. Nevertheless, there is still no similar study or research study about the substrate ratio between chicken waste leftover and rice waste leftover. Therefore, the correlation of substrate ratio between chicken waste leftover and rice waste leftover that has led into biogas production and even higher biohydrogen gas yield was further evaluated in this research. At the end of this study, the biogas mini-reactor was validated functioning and had potential in producing biohydrogen gases that has been successfully detected from the GC-TCD gas analysis test. This research was significantly important to be carried out as it provides another alternative for producing green biohydrogen alternative from food wastes resources which beneficial for sustainable and green environment.

## DEDICATION

*Dedicated to*

*My beloved father, Yip Cheok Yuen,*

*My appreciated mother, Wong Lai Peng,*

*My supervisor, Ts. Dr. Jeefferie Bin Abd Razak,*

*My appreciated families and all my friends and colleagues  
for giving me moral support, cooperation, encouragement and also understandings.*



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اونيورسيتي تيكنيكل مليسيا ملاك

UNIVERSITI TEKNIKAL MALAYSIA MELAKA

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# TABLE OF CONTENTS

|   |      |
|---|------|
| <b>ABSTRAK</b> .....  | i    |
| <b>ABSTRACT</b> .....   | ii   |
| <b>DEDICATION</b> .....   | iii  |
| <b>ACKNOWLEDGEMENT</b> .....  | iv   |
| <b>LIST OF TABLES</b> .....   | viii |
| <b>LIST OF FIGURES</b> .....  | xiv  |
| <b>LIST OF ABBREVIATIONS</b> .....  | xxiv |
| <b>LIST OF SYMBOLS</b> .....  | xxvi |
| <b>CHAPTER 1: INTRODUCTION</b> .....  | 1    |
| 1.1 Background.....   | 1    |
| 1.2 Problem Statement .....   | 3    |
| 1.3 Objectives.....   | 5    |
| 1.4 Scope .....   | 5    |
| 1.5 Justification of Studies .....  | 6    |
| 1.6 Summary of Methodology .....  | 7    |
| 1.7 Thesis Organization.....  | 9    |
| <b>CHAPTER 2: LITERATURE REVIEW</b> .....   | 10   |
| 2.1 Commercial Food Waste .....   | 10   |
| 2.1.1 Food Waste Definitions .....  | 11   |
| 2.1.2 Origin Sources of Food Waste.....   | 12   |
| 2.1.3 The impact of Movement Control Order (MCO) on food waste production caused by<br>Coronavirus (Covid-19) Outbreak..... | 13   |
| 2.1.4 Solid Waste Collection in Selangor.....   | 13   |
| 2.1.5 The impact of MCO on generation of total food waste in Selangor .....   | 14   |
| 2.1.6 The impact of MCO on the weekly generation of food waste in Selangor .....  | 15   |
| 2.1.7 The Influence of MCO to Food Waste Generation in Municipal Areas of Selangor.....                                     | 16   |
| 2.1.8 Impact of MCO on food waste generation in Selangor District Areas .....   | 17   |
| 2.2 Anaerobic Digestion (AD) .....  | 19   |
| 2.2.1 Important Stages of Anaerobic Digestion (AD) .....  | 19   |
| 2.2.2 The Anaerobic Digestion Process Quantitative Tests.....   | 21   |



|   |    |
|---|----|
| 2.2.3 Factor contributing to AD Device Failure .....                                    | 25 |
| 2.2.4 Co-digestion .....  | 26 |
| 2.3 Biogas Generation .....   | 26 |
| 2.3.1 Biochemical Reactions for Producing Biogas .....                                  | 27 |
| 2.3.2 Characteristic of Feedstock .....   | 28 |
| 2.3.3 Population of Livestock .....   | 30 |
| 2.3.4 Biogas Production Capacity.....   | 31 |
| 2.3.5 Biohydrogen .....   | 32 |
| 2.4 Reactor Design .....  | 35 |
| 2.4.1 Parameters in Reactor Operation .....   | 35 |
| 2.4.2 Applications of Biogas in Household Digesters .....                               | 37 |
| 2.4.3 Disadvantages .....   | 39 |
| 2.4.4 Environmental and Social Aspects of Biogas Digesters.....                         | 39 |
| 2.5 Previous Study Related with gas yield Quantification .....                          | 40 |
| 2.5.1 Quantification of Biohydrogen.....  | 40 |
| 2.5.2. Quantification using Gas Chromatography - Thermal Conductivity Detector (GC-TCD) | 43 |
| CHAPTER 3: METHODOLOGY .....  | 45 |
| 3.1 Introduction.....   | 45 |
| 3.2 Food Waste Collection and Feedstock Preparation .....                               | 47 |
| 3.2.1 Food Waste Collection and Planning .....  | 47 |
| 3.2.2 Food Waste Analysis .....   | 63 |
| 3.3 Biogas reactor Design.....  | 66 |
| 3.3.1 Schematic diagram .....   | 66 |
| 3.3.2 Materials and components required .....   | 67 |
| 3.3.3 Production Process.....   | 67 |
| 3.4 Output Quantification .....   | 68 |
| 3.4.1 Biohydrogen (H <sub>2</sub> ) Quantification .....                                | 68 |
| CHAPTER 4: RESULTS AND DISCUSSIONS .....  | 71 |
| 4.1 Overview .....  | 71 |
| 4.2 Feedstock Characterization.....   | 73 |
| 4.2.1 Fourier-transform Infrared Spectroscopy (FTIR).....                               | 73 |
| 4.3 Reactor design.....   | 88 |
| 4.3.1 Detail Drawing.....   | 88 |
| 4.3.2 Views of the product.....   | 89 |

|  |     |
|--|-----|
| 4.3.3 Exploded view .....  | 90  |
| 4.3.4 Product Components Functionalities.....                            | 91  |
| 4.3.5 Parts of product.....  | 93  |
| 4.3.6 Materials and components required .....                            | 99  |
| 4.3.7 Fabrication Process of Biogas Mini-Reactor.....                    | 99  |
| 4.4 GC-TCD Gas Analysis.....   | 100 |
| 4.4.1 GC-TCD results.....  | 100 |
| 4.4.2 FTIR results .....   | 113 |
| 4.5 Potential of Biohydrogen Production.....                             | 138 |
| 4.5.1 Comparison on mini reactor design for biohydrogen production ..... | 138 |
| 4.5.2 Potential of biohydrogen production and future outlook .....       | 140 |
| CHAPTER 5: CONCLUSIONS AND RECOMMENDATIONS .....                         | 145 |
| 5.1 Conclusions.....   | 145 |
| 5.2 Recommendations .....  | 146 |
| 5.3 Sustainability Element.....  | 146 |
| 5.4 Entrepreneur .....   | 146 |
| 5.5 Life Long Learning .....   | 147 |
| REFERENCES .....   | 148 |

## LIST OF TABLES

|     |   |    |
|-----|---|----|
| 2.1 | Food Waste Definition from various sources  | 11 |
| 2.2 | Period of data collection of solid waste in Selangor.   | 13 |
| 2.3 | List of selected local authorities in the processing of solid waste in Selangor.                            | 14 |
| 2.4 | Collection of food waste in Selangor prior to and during MCO.   | 15 |
| 2.5 | The different forms of inoculum used for the production of biogas from food waste                           | 24 |
| 2.6 | Factor contributing to Process Failure in AD System   | 25 |
| 2.7 | Composition of biogas (Baciocchi Renato, 2013)  | 28 |
| 2.8 | Comparison of biogas yield and electricity generated from various substrates of potential. (Stucki M, 2011) | 29 |
| 2.9 | Malaysian Livestock Population in 2012 (Federation of Malaysian Livestock Farmers' Association (FLFAM)).    | 30 |

|      |   |    |
|------|---|----|
| 2.10 | Impact on Process Efficiency of Co-Digestion of Protein-Rich Biomass with Carbon-Source Additives.                                  | 31 |
| 2.11 | Building materials for biogas reactor   | 36 |
| 4.1  | Basic information regarding band assignment for the stated range in Figure 4.1  | 75 |
| 4.2  | Some information regarding the peak position and chemical component in the chicken waste leftover sample.                           | 76 |
| 4.3  | Peak intensity information for carbohydrates, protein (amide I), amide (II) and fat component in the chicken waste leftover sample. | 79 |
| 4.4  | Basic information regarding band assignment for the range that stated in the Figure 4.6.  | 81 |
| 4.5  | Some information regarding the peak position and chemical component in the rice waste leftover sample.                              | 82 |
| 4.6  | Peak intensity information for carbohydrates, protein (amide I), amide (II) and fat   | 84 |

|      |   |     |
|------|---|-----|
|      | component in the rice waste leftover sample.  |     |
| 4.7  | Basic information regarding to the chicken waste leftover and rice waste leftover.        | 87  |
| 4.8  | Part name of biogas reactor   | 92  |
| 4.9  | Basic information for the graph analyses in Figure 4.24.                                  | 103 |
| 4.10 | One-point calibration for oxygen gas, nitrogen gas and carbon dioxide gas.                | 103 |
| 4.11 | Basic information for the graph analyses in Figure 4.25.                                  | 105 |
| 4.12 | One-point calibration for hydrogen gas, oxygen gas, nitrogen gas and carbon dioxide gas   | 105 |
| 4.13 | Basic information for the graph analyses in Figure 4.26                                   | 107 |
| 4.14 | One-point calibration for oxygen gas, nitrogen gas and carbon dioxide gas.                | 108 |
| 4.15 | Presence of gases   | 108 |
| 4.16 | The retention time of gases in minutes  | 109 |
| 4.17 | One-point calibration for hydrogen gases, oxygen gas, nitrogen gas and carbon dioxide gas | 110 |

|      |   |     |
|------|---|-----|
| 4.18 | The area of the peaks for gases detection in percentage (%).  | 111 |
| 4.19 | Total number of carbon (C) atom and hydrogen (H) atom for the chicken waste leftover and rice waste leftover sample.                    | 113 |
| 4.20 | Basic information regarding band assignment for the range that stated in the Figure 4.29.   | 115 |
| 4.21 | Some information regarding the peak position and chemical component in the chicken to rice ratio (1:1) sample.                          | 117 |
| 4.22 | Peak intensity information for carbohydrates, protein (amide I), amide (II) and fat component in the chicken to rice ratio (1:1) sample | 120 |
| 4.23 | Basic information regarding band assignment for the range that stated in the Figure 4.33.   | 122 |
| 4.24 | Some information regarding the peak position and chemical component in the chicken to rice ratio (1:2) sample.                          | 124 |
| 4.25 | Peak intensity information for carbohydrates, protein   | 127 |

|      |   |         |
|------|---|---------|
|      | (amide I), amide (II) and water component in the chicken to rice ratio (1:2) sample.  |         |
| 4.26 | Basic information regarding component for the range that stated in the Figure 4.38.   | 129     |
| 4.27 | Some information regarding the peak position and chemical component in chicken to rice ratio (2:1) sample                               | 130     |
| 4.28 | Peak intensity information for carbohydrates, protein (amide I), amide (II) and fat component in the chicken to rice ratio (2:1) sample | 134     |
| 4.29 | Basic information regarding to the chicken to rice ratio (1:1), chicken to rice ratio (1:2) and chicken to rice ratio (2:1).            | 137     |
| 4.30 | Total peak intensity for the chemical components of each sample.  | 137     |
| 4.31 | Comparison on the reactor design and the maximum biohydrogen production yield   | 139-140 |
| 4.32 | Percentage of hydrogen yield by each sample.  | 143     |





## LIST OF FIGURES

|     |  |    |
|-----|--|----|
| 1.1 | Overall research flow  | 8  |
| 2.1 | Sorting of municipal solid waste (MSW) for materials recycling and energy recovery                   | 12 |
| 2.2 | Weekly Food Waste Collection in Selangor (19 February 2020-14 April 2020)                            | 16 |
| 2.3 | Weekly food waste collection contrast between municipal areas in Selangor before and during the MCO. | 17 |
| 2.4 | Weekly collection of food waste from the Selangor district areas.                                    | 18 |
| 2.5 | Production of biogas from food waste through the process of anaerobic digestion (AD).                | 20 |
| 2.6 | Biogas generation cycle (G, Northrup .2015)  | 27 |
| 2.7 | The total contribution to the production of biogas from the primary source of organic waste.         | 29 |

|     |   |    |
|-----|---|----|
| 2.8 | Methods of biogas collecting<br>(A: gasbags, B: helium<br>balloon, C: syringes)<br>(Fatameh, 2020).   | 41 |
| 3.1 | Flow chart of overall<br>methodology  | 46 |
| 3.2 | A fast food outlet worker was<br>sort out the food waste<br>(chicken leftover, bun<br>leftover, potato leftover and<br>coleslaw leftover) from<br>garbage bag for the first day<br>of collection. | 47 |
| 3.3 | Chicken leftover (Meat and<br>bones) were sorted out from<br>the garbage bag.   | 48 |
| 3.4 | Chicken leftover (Bones and<br>meats) were seperated<br>accordingly in two different<br>medium.   | 48 |
| 3.5 | Meats from chicken leftover<br>were rinsed with tap water<br>and shake well after rinsed.   | 49 |
| 3.6 | Chicken meats were dried for<br>150c, 40 minutes for two<br>times in the oven.  | 49 |
| 3.7 | Chicken meats is then dried<br>under the sun until it is<br>completely dried.   | 50 |
| 3.8 | Dried chicken meat in the<br>powder form.   | 50 |

|      |  |    |
|------|--|----|
| 3.9  | A 369 grams of dried chicken powder and tap water respectively.                          | 51 |
| 3.10 | A 738 grams of dried chicken powder and tap water.                                       | 51 |
| 3.11 | A 184.5 grams of dried chicken powder and tap water.                                     | 52 |
| 3.12 | Rice leftover that collected from household.   | 52 |
| 3.13 | Dried rice leftover that have dried completely from oven.                                | 53 |
| 3.14 | Dried rice leftover was blended by using blender.  | 53 |
| 3.15 | Rice powder form.  | 54 |
| 3.16 | A 369 grams of rice powder and tap water respectively.                                   | 54 |
| 3.17 | A 738 grams of rice powder and tap water.  | 55 |
| 3.18 | A 184.5 grams of rice powder and tap water.  | 55 |
| 3.19 | Substrate ( Chicken leftover and rice leftover) was poured into the reactor accordingly. | 56 |
| 3.20 | Fresh cow dung has collected by using a shovel.  | 57 |
| 3.21 | Plastic bucket that contains seven litres of fresh cow dung.                             | 57 |

|      |   |    |
|------|---|----|
| 3.22 | Fermented cow dung that appeared army green in colour.  | 58 |
| 3.23 | A seven litres of tap water were added into 7 litres of fermented cow dung.   | 58 |
| 3.24 | A seven litres of tap water and 7 litres of fermented cow dung were stirred by using a steel stick.                   | 59 |
| 3.25 | A 14 litres of cow dung slurry.   | 59 |
| 3.26 | 500 grams of cow dung slurry that marked by an orange strip   | 60 |
| 3.27 | Cow dung slurry that was prepared to inserted inside to the digester.   | 60 |
| 3.28 | Three digesters with the chicken to rice ratio of 2:1, 1:1 and 1:2 was placed in the polymer lab in FKP old building. | 61 |
| 3.29 | Overall feedstock preparation in this study.  | 62 |
| 3.30 | FTIR spectrophotometer (JASCO)  | 63 |
| 3.31 | Dried chicken waste leftover and dried rice waste leftover for the FTIR analysis                                      | 64 |

|      |  |    |
|------|--|----|
| 3.32 | ATR sample setting in FTIR spectrometer.   | 64 |
| 3.33 | Sample that undergoes FTIR analysis.   | 65 |
| 3.34 | FTIR analysis result for the following sample is analyzed.   | 65 |
| 3.35 | Schematic diagram for biogas reactor tank  | 66 |
| 3.36 | GC-TCD gas analysis was performed at the material characterization lab of Universiti Putra Malaysia. | 68 |
| 3.37 | Gas Chromatography-Thermal Conductivity Detector (GC-TCD) (Model 6890-N)                             | 69 |
| 3.38 | The sample that filled with biogas is being injected by a syringe.                                   | 69 |
| 3.39 | The syringe that filled with one millilitre of the sample is being injected into a septum            | 70 |
| 3.40 | The compound of the sample is then being record.   | 70 |
| 4.1  | Fourier-transform Infrared Spectroscopy (FTIR) analysis for Chicken Waste Leftover.                  | 74 |

|     |   |    |
|-----|---|----|
| 4.2 | Peak position for carbohydrates, protein (amide I), protein (amide II), fat and water in the chicken waste leftover sample. | 76 |
| 4.3 | Structure of Chicken waste leftover   | 77 |
| 4.4 | Deep fried chicken  | 77 |
| 4.5 | Peak intensity for carbohydrates, protein (amide I), amide (II) and fat component in the chicken waste leftover sample.     | 79 |
| 4.6 | Fourier-transform Infrared Spectroscopy (FTIR) analysis for Rice Waste Leftover.  | 81 |
| 4.7 | Peak position for carbohydrates, protein (amide I), protein (amide II), fat and water in the rice waste leftover sample.    | 82 |
| 4.8 | Peak intensity for carbohydrates, protein (amide I), amide (II) and fat component in the rice waste leftover sample.        | 84 |
| 4.9 | Comparison between chicken waste leftover and rice waste leftover.  | 86 |

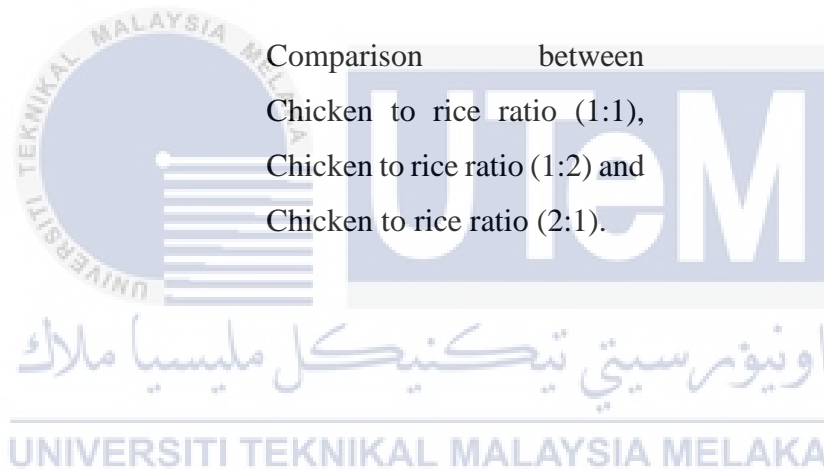
|      |   |     |
|------|---|-----|
| 4.10 | Detail drawing for the biogas mini-reactor  | 88  |
| 4.11 | Assembly view of biogas mini-reactor in orthographic view   | 89  |
| 4.12 | Exploded view of biogas mini-reactor in orthographic view.  | 90  |
| 4.13 | Inlet pipe  | 93  |
| 4.14 | On off valve  | 93  |
| 4.15 | Outlet pipe   | 94  |
| 4.16 | Pipe plug   | 94  |
| 4.17 | Plastic container cap   | 95  |
| 4.18 | Plastic tubing 280 mm   | 95  |
| 4.19 | Plastic tubing 300 mm   | 96  |
| 4.20 | Plastic tubing 380 mm   | 96  |
| 4.21 | Plastic tubing 400 mm   | 97  |
| 4.22 | T connector   | 97  |
| 4.23 | Tyre tube   | 98  |
| 4.24 | Gas Chromatography-Thermal Conductivity Detector (GC-TCD) gas analysis for Chicken to rice ratio (1:1). | 102 |
| 4.25 | Gas Chromatography-Thermal Conductivity Detector (GC-TCD) gas analysis for Chicken to rice ratio (1:2). | 105 |

|      |   |     |
|------|---|-----|
| 4.26 | Gas Chromatography-<br>Thermal Conductivity<br>Detector (GC-TCD) gas<br>analysis for Chicken to rice<br>ratio (2:1).  | 107 |
| 4.27 | Chemical molecular structure<br>for the rice waste leftover.  | 112 |
| 4.28 | Chemical molecular structure<br>for the chicken waste<br>leftover.  | 113 |
| 4.29 | Fourier-transform Infrared<br>Spectroscopy (FTIR) analysis<br>for chicken to rice ratio (1:1)<br>sample.  | 115 |
| 4.30 | Peak position for<br>carbohydrates, protein<br>(amide I), protein (amide II),<br>fat and water in the chicken to<br>rice ratio (1:1) sample.  | 117 |
| 4.31 | Dried rice waste leftover<br>(greyish colour), chicken<br>waste leftover (brownish<br>colour) and cow dung<br>inoculum (charcoal black<br>colour) in chicken to rice<br>ratio (1:1) sample. | 119 |
| 4.32 | Peak intensity for<br>carbohydrates, protein<br>(amide I), amide (II) and fat<br>component in the chicken to<br>rice ratio (1:1) sample   | 120 |



|        |   |     |
|--------|---|-----|
| 4.33   | Fourier-transform Infrared Spectroscopy (FTIR) analysis for chicken to rice ratio (1:2) sample.   | 122 |
| 4.34   | Peak position for carbohydrates, protein (amide I), protein (amide II), fat and water in the chicken to rice ratio (1:2) sample.  | 123 |
| 254.35 | Molecular structure of glucose  | 125 |
| 4.36   | Dried rice waste leftover (yellowish colour), chicken waste leftover (brownish colour) and cow dung inoculum (charcoal black colour) in chicken to rice ratio (1:2) sample. | 125 |
| 4.37   | Peak intensity for carbohydrates, protein (amide I), amide (II) and water component in the chicken to rice ratio (1:2) sample.  | 126 |
| 4.38   | Fourier-transform Infrared Spectroscopy (FTIR) analysis for chicken to rice ratio (2:1) sample.   | 128 |
| 4.39   | Peak position for carbohydrates, protein (amide I), protein (amide II),   | 130 |

|      |  |     |
|------|--|-----|
|      | fat and water in the chicken to rice ratio (2:1) sample.   |     |
| 4.40 | Dried chicken waste leftover (brownish colour), rice waste leftover (greyish colour) and cow dung inoculum (charcoal blackish colour). | 132 |
| 4.41 | Peak intensity for carbohydrates, protein (amide I), amide (II) and fat component in the chicken to rice ratio (2:1) sample.           | 133 |
| 4.42 | Comparison between Chicken to rice ratio (1:1), Chicken to rice ratio (1:2) and Chicken to rice ratio (2:1).                           | 136 |



## LIST OF ABBREVIATIONS

|                  |   |  |
|------------------|---|--|
| CO               | - | Carbon monoxide                                  |
| H <sub>2</sub>   | - | Hydrogen   |
| CO <sub>2</sub>  | - | Carbon dioxide                                   |
| CH <sub>4</sub>  | - | Methane  |
| H <sub>2</sub> S | - | Hydrogen sulphide                                |
| VS/TS            | - | Volatile solids/total solids                     |
| pH               | - | Potential of hydrogen                            |
| C/N ratio        | - | Carbon to nitrogen ratio                         |
| HRT              | - | Hydraulic retention time                         |
| FTIR             | - | Fourier Transform Infrared Spectroscopy          |
| S/I              | - | Substrate to inoculums ratio                     |
| MSW              | - | Municipal solid waste                            |
| AD               | - | Anaerobic digestion                              |
| KRI              | - | Khazanah research institute                      |
| MCO              | - | Movement Control Order                           |
| VFA              | - | Volatile fatty acid                              |
| GHG              | - | Greenhouse gases                                 |
| LCFA             | - | Long chain fatty acid                            |
| OLR              | - | Organic loading rate                             |
| GC-TCD           | - | Gas chromatography-thermal conductivity detector |
| WHO              | - | World Health Organization                        |
| DVS              | - | Department of Veterinary Services                |
| PVC              | - | Poly-vinyl chloride                              |
| CSTR             | - | Continuous Stirred Tank Reactor                  |
| LPG              | - | Liquefied Petroleum Gas                          |
| FID              | - | Flame-ionization detection                       |
| FKP              | - | Faculty Kejuruteraan Pembuatan                   |
| ATR              | - | Attenuated Total Reflectance                     |
| UPM              | - | Universiti Putra Malaysia                        |
| STR              | - | Stirred Tank Reactor                             |

|       |   |   |
|-------|---|---|
| UASB  | - | Upflow Anaerobic Sludge Blanket Reactor |
| ASBR  | - | Anaerobic Sequencing Batch Reactor      |
| TBSBR | - | Trickling Bed Sequenced Batch Reactor   |
| FBR   | - | Fluidized Bed Reactor                   |



## LIST OF SYMBOLS

|                |   |                   |
|----------------|---|-------------------|
| %              | - | Percent           |
| m <sup>3</sup> | - | cubic meter       |
| kPa            | - | kilopascal        |
| °C             | - | Degree Celsius    |
| ppm            | - | parts per million |
| kW.h           | - | kilowatt hour     |
| MW             | - | Megawatt          |
| V              | - | Volt              |
| Kg             | - | Kilogram          |
| TWh            | - | Terawatt-hour     |



# CHAPTER 1

## INTRODUCTION

### 1.1 Background

High expense of generating biohydrogen as a commercially viable source of energy for combustion engines and fuel cells are becoming of major problem. Although hydrogen production is currently expensive, it was expected to play an important role in the future energy economy. Traditional methods, such as thermo catalytic conversion and electrolysis, have been shown to be both energy and cost-intensive (Levin, 2004). In this situation, biological hydrogen generation may be able to overcome some of the economic constraints of conventional above-said production method. The utilisation of food waste and industrial effluents for biohydrogen production had potentially reducing the hydrogen production costs.

On the other hand, anaerobic digestion as biological route is remarkably suitable choice for alternative production for renewable energy harvested from food waste source, since food waste is mostly composed of organic components. According to Braun, the resultant biogas is mostly composed of methane,  $\text{CH}_4$  (55-70 %), carbon dioxide,  $\text{CO}_2$  (30-45 %), hydrogen,  $\text{H}_2$  (22-34 %), and lesser amounts of hydrogen sulphide,  $\text{H}_2\text{S}$  (50-2000 ppm), water vapour, oxygen, and other hydrocarbon traces. According to Pedro et al. (2017), hydrogen ( $\text{H}_2$ ) may be utilised as a renewable energy source for electricity production and power facilities. Among these technologies, Koleva (2011) had stated that anaerobic digestion has the greatest potential to convert food waste into renewable energy since it offers many environmental benefits, such as the creation of renewable energy network, the

ability to recover nutrients, and the decrease of waste volumes. As a result, the potential characteristics of this chosen technology was evaluated further in this study.

Thus far, it has been known that the food waste is an under-utilised resource with significant potential for energy generation. According to McKendry (2002), there are numerous biomass-to-energy conversion methods, but there has been no special emphasis on the use of food waste as a feedstock. Food waste as a feedstock for anaerobic digestion is gaining popularity due to the potential energy harvesting. Ohkouchi and Inoue (2007) has identified food waste with higher quantities of organic matter (volatile solids/total solids [VS/TS]: 0.8–0.9, higher moisture content, and great biodegradability as the most promising anaerobic substrates. According to Labatut (2011), food waste is heavy in lipids (animal fats, used oil, and ice cream) and readily degradable carbohydrates (rice and potatoes) may provide a significant biohydrogen output. Food waste, on the other hand, with higher lignocellulosic component and lower lipid content, such as fruit and vegetable leftovers and brewing waste, has lower potential of biohydrogen production of approximately 0.16-0.35 m<sup>3</sup>. As a result, in this research, chicken leftovers with higher protein content and rice leftovers with an easily degradable carbohydrate content were completely used as food waste sources of substrate feedstock.

In Malaysia, Md. Zayed (2013) had stated that cows producing 55 litres of cow dung on average per day. It would create heavy waste production which environmentally damaging. This has evident when cow dung becomes slurry, which combines animal excrement with rainwater, wash water, and, in certain instances, waste bedding and feed are some causes of water and air pollution. Water pollution from overflowing slurry storage or run-off from heavy rain may damage fish and aquatic life in freshwater systems by raising biochemical oxygen demand (BOD), dissolved ammonia, and phosphorus levels, resulting in algal blooms (Md. Zayed, 2013). Air pollution has releasing ammonia gas into the atmosphere from manures spread on the field and stored in animal barns. Anaerobic digestion is a waste-to-energy method that is widely used to handle a variety of organic wastes. Examples include the organic component of municipal solid waste, sewage sludge, food waste, and animal manure. So far, the present study trend has mostly focused on the area of pH and temperature in producing hydrogen. Nonetheless, there are no comparable

study in evaluating the relationship between the substrate ratio of food waste (chicken leftover and rice leftover waste) with fixed content of cow dung. Parameters such as substrate ratio of food waste (chicken waste and rice waste leftover) could be adjusted to provide the best media for microorganism's activities and therefore enhancing biohydrogen production. It was anticipated that by increasing the substrate content of rice leftover waste might increase biohydrogen gas production. As a result, it was anticipated that by correlating the substrate ratio of chicken leftover and rice leftover waste, the biohydrogen gas output would be increased further, as opposed to the correlation of pH and temperature, which is widely accessible in literature. However, due to time and facilities constraints (COVID-19 and previous MCO), the biohydrogen production in this research has mainly emphasised to the correlation effect between the substrate ratio of chicken leftover and rice leftover waste, towards the detection of biohydrogen, using GC-TCD analytical equipment. In addition, the potential of mini-reactor for anaerobic digestion of bio-hydrogen production was evaluated further in this work.



## 1.2 Problem Statement

Up until now, research has focused on the use of food waste for biogas production. However, disintegrating food waste sources in order to get the satisfied yield of biogas output was very challenging. As foaming may occur without mixing, and too much mixing would stress the microbe, combining various food waste sources may also play a crucial effect. Careful selection of two or more distinct kinds of feedstock (Lvarez, 2010) is required to improve the effectiveness of anaerobic digestion. Furthermore, Sommer (2008) said that the dry matter content is a critical element in the design and capabilities of the biogas mini reactor. Variety of food waste sources would result in excessive moisture content, causing the biogas digester to malfunction. As a result, considerable preliminary work has to be done in order to reach a definitive conclusion on defining two kinds of food waste, which are, in this research, chicken waste leftover and rice waste leftover.

However, until recently, there has been a dearth of knowledge about biogas production in Malaysia. This is shown by the fact that biogas is projected to produce 100



MW of power by 2021 (Shafie, 2021), with a 410 MW energy reserve by 2030 and 360–400 MW by 2021. (Khor CS, 2021). However, the Malaysia Sustainable Energy Development Authority (SEDA) reports that the total installed capacity for biogas is only 6.48 MW (from landfill/agricultural waste) and 6.36 MW (from landfill/agricultural waste) by 2021. There is undeniably increasing worry about the high rate of organic waste generation as a consequence of fast urbanisation and population increase nowadays. It was regarded as a serious issue for environmental preservation because foul odours, toxic leachate, and greenhouse gas emissions would arise as a result of incorrect disposal of food waste with high moisture content into landfills. As a result, it was beneficial to fully use food waste as a feedstock for anaerobic digestion (AD) for biogas generation. Food waste is considered as readily biodegradable organic substrate due to its high moisture content. Furthermore, during the MCO period in Malaysia, the proportion of household waste produced by over-purchasing, particularly food waste, may have risen owing to the increased frequency of operations at home as a consequence of stay-at-home orders. The residential sector accounts for 44.5 % of total solid waste collection results, or 6.1 million tonnes per year, according to the Khazanah Research Institute (KRI). Apart from that, cow dung is no exception, since it emits a harmful greenhouse gas known as carbon dioxide, which adds to the greenhouse effect. This issue will be addressed if cow dung is converted into biofuel for use in the anaerobic digestion process. As a result, in order to address this problem, food waste was employed as a substrate, and cow dung was used as inoculum in this study.

UNIVERSITI TEKNIKAL MALAYSIA MELAKA

Besides that, the creation of hydrogen sulphide ( $H_2S$ ), rather than methane ( $CH_4$ ) and biohydrogen ( $H_2$ ), is the element that we do not want in biogas production. According to Wellinger and Linberg (2011),  $CH_4$  must be removed at a rate more than 95% and  $H_2S$  must be removed at a rate less than 4 ppm (2000). As a result, one of the current problems facing industry is in determining ways to decrease  $H_2S$  production. Industrial competitors recommended that we have a longer digesting period for food waste in order to minimise  $H_2S$ . By conducting this research, it was demonstrated that the substrate ratio was an essential component in biogas production that may decrease the  $H_2S$  concentration and improve biohydrogen output. However, owing to facilities constraints,  $H_2S$  quantification is not possible. In contrast, the gas chromatography-thermal conductivity detector (GC-TCD) equipment was utilised and the detection of biohydrogen gases was becoming as the primary parameter in this study. The detection of biohydrogen gases was the goal of this study. To

date, no systematic research has been performed to investigate the effect of substrate ratio (chicken leftover and rice leftover waste) with fixed content of cow dung inoculum, and, more significantly, on their relationship. It is a critical issue to consider in industry since the substrate ratio of food waste could affect the profit creation.

In short, by examining all of the above factors, the reason for doing this study is clearly stated and warrants further investigation. This is due to the fact that no prior related study, particularly regarding the correlation between the substrate ratio of chicken leftover and rice leftover waste with the fixed amount of inoculum, has been reported in the present literature.

### 1.3 Objectives

The objectives of the reported study are as follows:

- (a) To characterize the specific food waste (chicken leftover and rice leftover) in terms of their chemical composition by using Fourier transform infrared (FTIR) analysis.
- (b) To evaluate the effects of substrate (Chicken to rice leftover ratio) feedstock with fixed content of inoculum during anaerobic digestion.
- (c) To evaluate the potential of the hydrogen production using proposed mini-reactor design.

### 1.4 Scope

The research scopes are as follows:

- (a) Collection of commercial food waste sources (chicken leftover and rice leftover) in one popular fast-food outlet in Gangsa, Melaka.

- (b) Characterize the chemical composition of chicken leftover and rice leftover waste through Fourier transform infrared (FTIR) analysis.
- (c) Design mini reactor for anaerobic digestion process content of maximum five to ten litre of substrate and inoculum.
- (d) Evaluate the potential of substrate between chicken waste leftover and rice waste leftover without evaluating the single influence of chicken waste leftover and rice waste leftover.
- (e) Analyse biohydrogen gas (H<sub>2</sub>) yield by using gas chromatography-thermal conductivity detector (GC-TCD).
- (f) Evaluating the potential of biohydrogen production using proposed mini-reactor design.

### 1.5 Justification of Studies

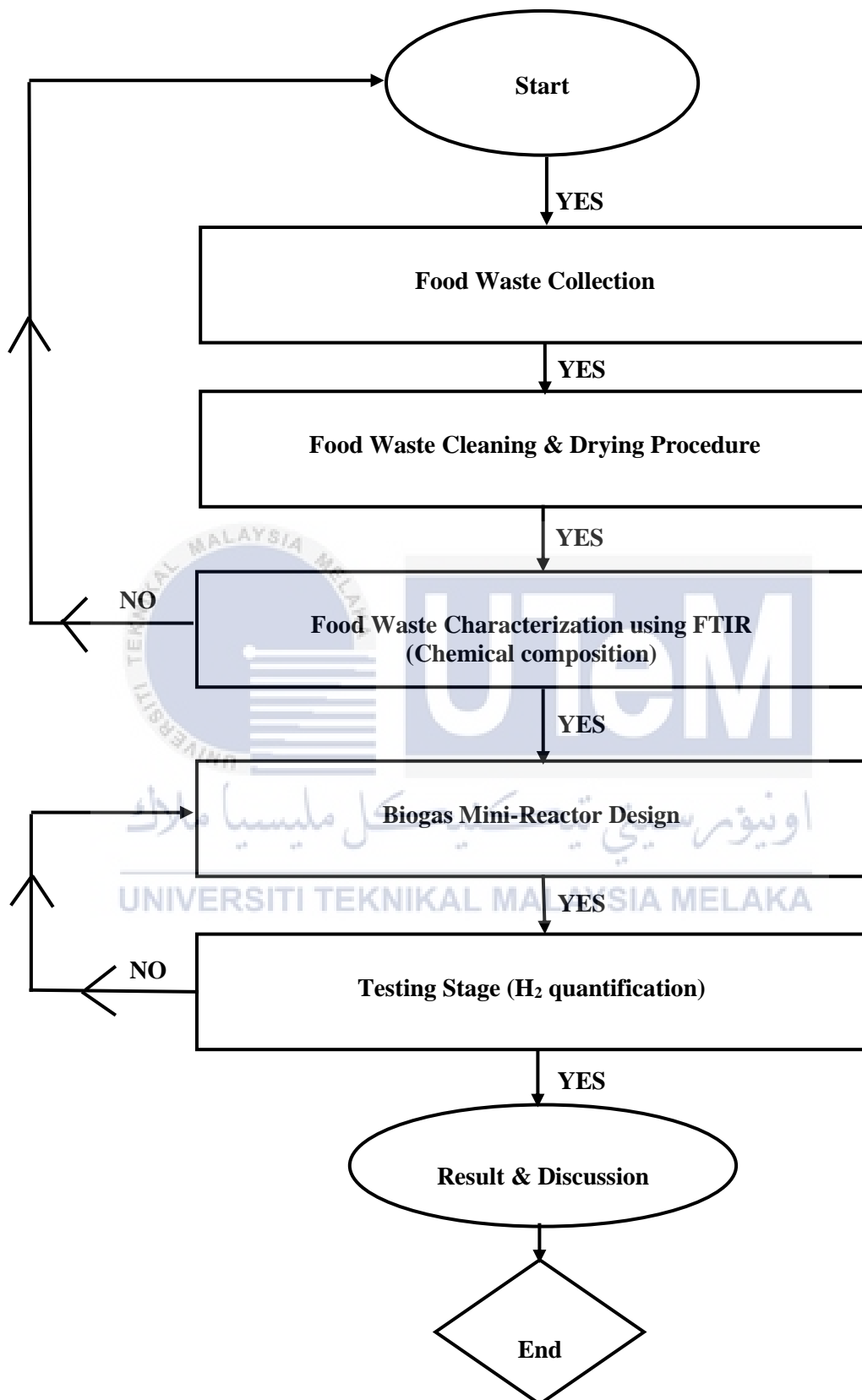
The study rationales are explained in depth as follows:

- (a) Biohydrogen gas yield from commercial food waste source would be developed from this study by using proposed mini reactor design.
- (b) Develop more information and also deep understanding about the correlation of substrate ratio between the chicken leftover and rice leftover waste with fixed content of inoculum as to improve the biohydrogen yield production.
- (c) There are certain potential benefits that country and industry may benefit from the completion of this report especially on alternative green energy production from waste resources.

## 1.6 Summary of Methodology

The overall research flow was summarized as in Figure 1.1. At beginning of the flow, the research was started with food waste collection in one popular fast-food outlet located at Gangsa, Melaka. After that, the feedstock was undergoing preparation and conditioning by cleaning and drying method. Next, the food waste was undergoing the characterization to determine their chemical composition through Fourier Transform Infrared (FTIR) analysis. Next, design and fabrication of biogas mini-digester tank was carried out. After that, the testing stage for biohydrogen detection was conducted. Lastly, the conclusion was made based on the conducted works and findings.

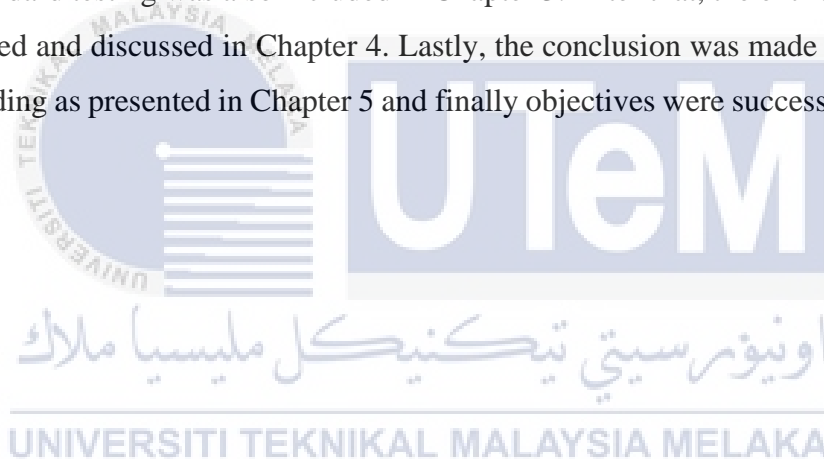




**Figure 1.1:** Overall research flow

## 1.7 Thesis Organization

The thesis arrangement was started with the Chapter 1. This chapter has explored the study's context. Via observations, issues are established. This is accompanied by targets to be accomplished during the analysis and scope that narrows down the study field. Next, Chapter 2 was followed. This chapter covers the basic theories about the research topic and previous journal and information gathering. The methods for evaluating biohydrogen production system were described. The Chapter 3 was the methodology that describes all raw used and also related process applied to produce the biohydrogen gas from commercial food waste source (chicken leftover and rice leftover) and inoculum (cow dung). Other than that, the standard testing was also included in Chapter 3. After that, the entire data collected were analysed and discussed in Chapter 4. Lastly, the conclusion was made based from the analysis finding as presented in Chapter 5 and finally objectives were successfully achieved.



## **CHAPTER 2**

### **LITERATURE REVIEW**

This chapter provides overview on anaerobic digestion of biogas production from the commercial food waste source for green energy generation. Commercial food waste was reviewed at the first part of this chapter. Besides, current situation was also discussed. Other than that, information related to anaerobic digestion. Last but not least, related previous study on biogas quantification was explored and reviewed in this chapter.

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#### **2.1 Commercial Food Waste**

This section was mainly focused on the review of commercial food waste. Current situation that led into increasing amount of commercial food waste generated was covered with details under this section. Besides, the sources for food waste were also summarized in this chapter.

### 2.1.1 Food Waste Definitions

Food waste definitions are not generally agreed upon, making related study and measurement of food waste are challenging (Buzby and Hyman, 2012). Different categorizations of food waste are created based on the materials, methods of manufacturing, and management techniques. (Gjerris and Gaiani, 2013). Several words have been used interchangeably, including food loss, food waste, bio waste, and kitchen trash (Schneider, 2013a). Similar terms are often used, but with different meanings (Gjerris and Gaiani, 2013). This is exacerbated by report translation (Schneider, 2013a). Table 2.1 provides an overview of previously used definitions of food waste.

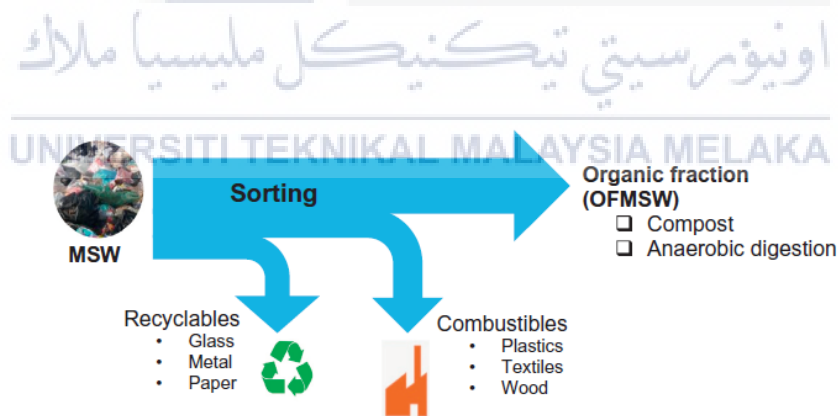
**Table 2.1:** Food Waste Definition from various sources

| Source  | Year | Definition  |
|---|------|---|
| Food and Agriculture Organization (FAO)                             | 2013 | Food waste is food discarded for consumption by humans (generally at retail and consumption stages).  |
| European Commission   | 2014 | Food waste is food that has been lost in the food supply chain (including inedible parts), not including food that has been diverted into material uses, such as bio-based products, animal feed, or sent for redistribution.   |
| United States Environmental Protection Agency (USEPA)               | 2014 | Food waste from domestic, industrial, and institutional facilities is unconsumed food and food preparation waste. Therefore, food waste from homes, grocery shops, restaurants, bars, factory lunchrooms, and corporate cafeterias are all covered. Pre-consumer food waste generated during the manufacturing and packaging of foodstuffs is not included. |
| United States Department of Agriculture (USDA) (Buzby et al., 2014) | 2014 | Food waste is a subset of food loss which occurs when there is no consumption of an edible object. Waste is only known to be food that is still edible at the time of disposal.   |
| World Resources Institute (WRI)                                     | 2015 | Food loss and waste applies to food diverted from the food supply chain, as well as the associated inedible parts.  |



### 2.1.2 Origin Sources of Food Waste

Food waste is food that is safe to consume but is wasted due to deterioration. Lower-income nations allegedly lost food throughout the manufacturing phase, whereas medium and high-income countries lost food during the last phase of the home phase. Based on USDA (United States Department of Agriculture), food waste in Malaysia is produced in rural, industrial, institutional, commercial, and urban locations. Food waste from retailers were also disposed of as urban solid waste (MSW). Food waste is the most prevalent component of MSW, accounting for 20-54 % of trash generated in different nations (Yasin, 2013). Because of lower quality and present of contaminants, the recycling rate of food waste found in MSW is lower than that found in commercial food waste. Sorting of MSW is just as essential for anaerobic digestion (AD) as it is for other processes like composting. MSW sorting may be done at the source or at central sorting facilities. Overall, AD is a promising technique for recovering and treating food waste from resources (Xu, 2018). Figure 2.1 depict the sorting of MSW for materials recycling and energy recovery, which classified food waste under the organic fraction MSW (OFMSW).



**Figure 2.1:** Sorting of municipal solid waste (MSW) for materials recycling and energy recovery

### 2.1.3 The impact of Movement Control Order (MCO) on food waste production caused by Coronavirus (Covid-19) Outbreak

Coronavirus illness, known as COVID-19 by the World Health Organization (WHO), is spreading quickly in almost all areas of Asia, as well as most nations in Europe and North America (Bhusare, 2020). The Malaysian government announced the introduction of Movement Control Order (MCO) as part of strategy to flatten the pandemic curve (Salim, 2020). The increasing number of home operations as a result of stay-at-home would significantly raise the percentage of household waste generated by over-buying or unplanned order. Households are the primary source of municipal solid trash in Malaysia. According to Khazanah Research Institute (KRI), the residential sector accounts for 44.5 % of overall solid waste collection, with 6.1 million tonnes was generated for each year. MSW consists of 20 distinct kinds of waste, including food waste, which accounts for 50% of total waste composition (Sundaram, 2019).

### 2.1.4 Solid Waste Collection in Selangor

The Project Delivery Group, KDEB Waste Management, has conducted the Domestic Waste Collection data across Selangor on a regular basis from 19 February 2020 to 14 April 2020 (Table 2.2) to assess relative changes of MSW via a system of waste weighing.

**Table 2.2:** Period of data collection for solid waste in Selangor.

| Collection Period        | Collection Duration | Abbreviation |
|--------------------------|---------------------|--------------|
| One weeks before MCO     | 11–17 March 2020    | 1WB MCO      |
| Week 1 of Phase 1 of MCO | 18–24 March 2020    | W1 P1 MCO    |
| Week 2 of Phase 1 of MCO | 25–31 March 2020    | W2 P1 MCO    |
| Week 1 of Phase 2 of MCO | 1–7 April 2020      | W1 P2 MCO    |
| Week 2 of Phase 2 of MCO | 8–14 April 2020     | W2 P2 MCO    |

The collection of solid waste in Selangor covers total of 12 local authorities and was classified by their requirements and, as shown in Table 2.3, can be easily identified by its name.

**Table 2.3:** List of selected local authorities in the processing of solid waste in Selangor.

| City Council                          | Municipal Council                     | District Council                    |
|---------------------------------------|---------------------------------------|-------------------------------------|
| Majlis Bandaraya Petaling Jaya (MBPJ) | Majlis Perbandaran Ampang Jaya (MPAJ) | Majlis Daerah Kuala Langat (MDKL)   |
| Majlis Bandaraya Shah Alam (MBSA)     | Majlis Perbandaran Kajang (MPKj)      | Majlis Daerah Sabak Bernam (MDSB)   |
|                                       | Majlis Perbandaran Klang (MPK)        | Majlis Daerah Hulu Selangor (MDHS)  |
|                                       | Majlis Perbandaran Selayang (MPS)     | Majlis Daerah Kuala Selangor (MDKS) |
|                                       | Majlis Perbandaran Sepang (MPSp)      |                                     |
| Majlis Perbandaran Subang Jaya (MPSJ) |                                       |                                     |

### 2.1.5 The impact of MCO on generation of total food waste in Selangor

Table 2.4 shows the overall build-up of food waste in Selangor before and after the MCO. The overall amount of food waste produced during the collecting period is 104,201 tonnes, with a standard deviation of 1008.4 and a sample variance of 1,016,955.1. The collection of food waste in the four weeks before the MCO's announcement of a secure minimal relative waste trend. One week before the MCO, 13,927.44 tonnes of food waste were recorded throughout Selangor. A proportional decrease of 7.88 percent to a total of up to 12,830.48 tonnes of food waste was recorded one week following the MCO. In the second week of MCO, about 14.76 % reduction in 11,871.70 tonnes of food waste was recorded.

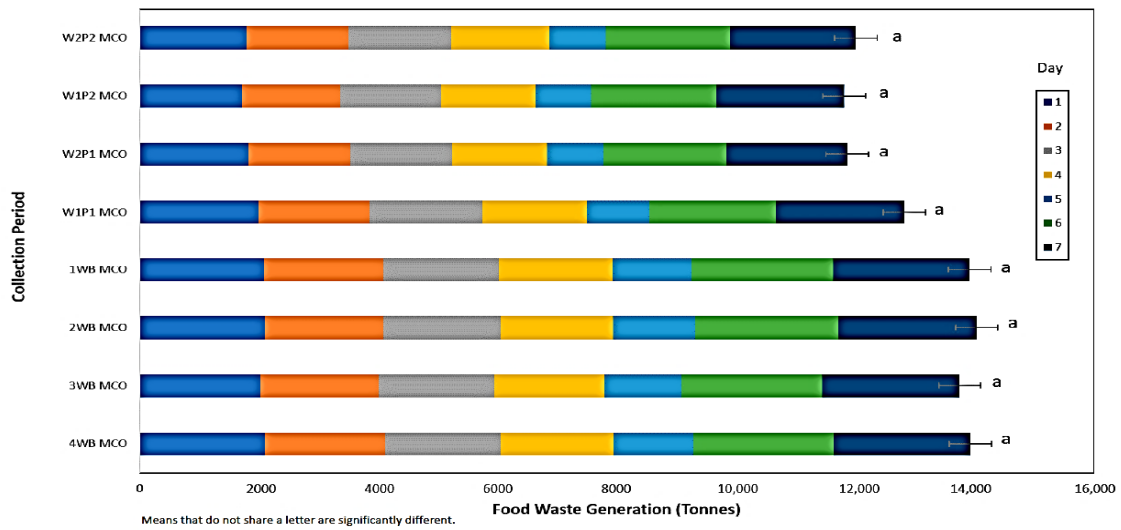
The pandemic has caused commercial and industrial activities to temporarily close, forcing the majority of individuals to stay at home (Marked Drop in Waste Sent to Landfill, 2020).

**Table 2.4:** Collection of food waste in Selangor prior and during MCO (Latifah Abd Manaf, 2020).

| Period                 | 4WB<br>MCO         | 3WB<br>MCO | 2WB<br>MCO | 1WB<br>MCO | W1 P1<br>MCO | W2 P1<br>MCO | W1 P2<br>MCO | W2 P2<br>MCO |
|------------------------|--------------------|------------|------------|------------|--------------|--------------|--------------|--------------|
| Food waste<br>(tonnes) | 13,938.0           | 13,757.4   | 14,040.8   | 13,927.4   | 12,830.5     | 11,871.7     | 11,820.7     | 12,015.3     |
| Relative change<br>(%) | Reference<br>value | -1.3       | 0.75       | -0.08      | -7.95        | -16.10       | -17.83       | -16.27       |

### 2.1.6 The impact of MCO on the weekly generation of food waste in Selangor

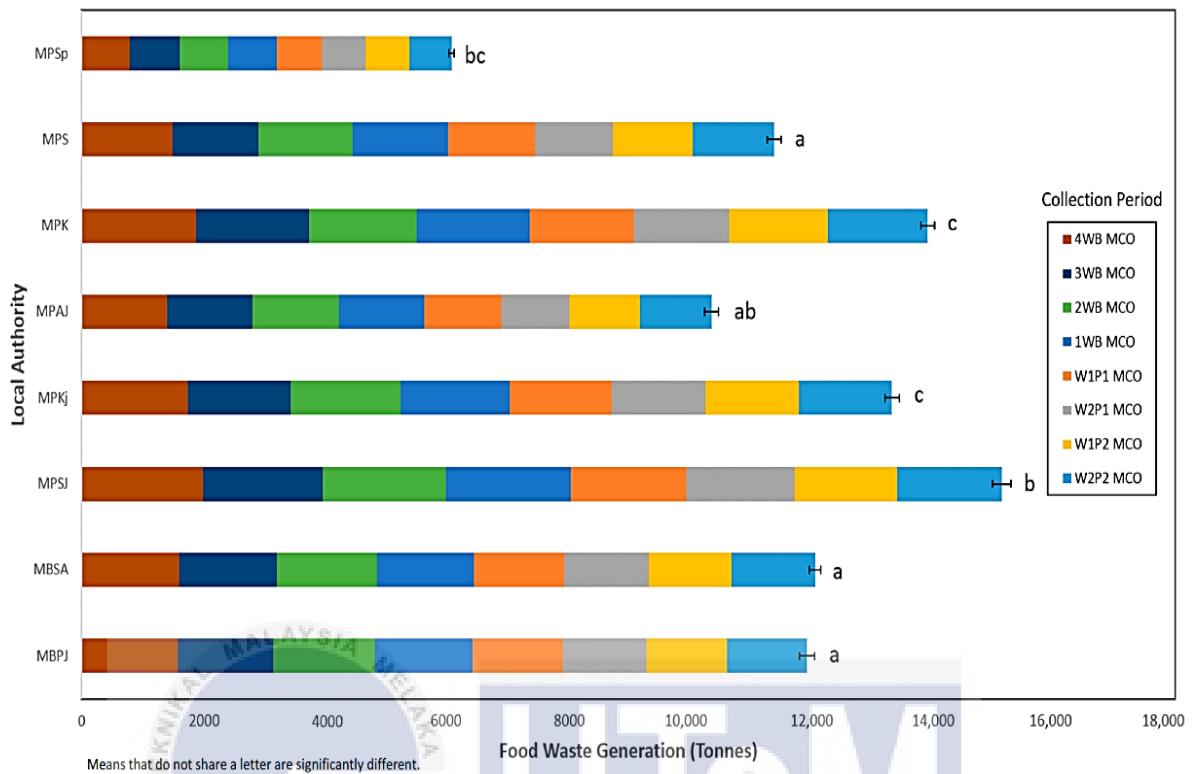
Figure 2.2 depicts the collection of food waste for four weeks period before MCO and the first four weeks of MCO. The graphic demonstrated (and indicated with a similar letter a) that there were no statistically significant differences ( $p=0.05$ ) between food waste collection in Selangor and the weekly collection period. Selangor recorded 2083.86, 1999.37, 1939.96, 1908.69, 1329.21, 2370.07, and 2296.27 tonnes of food waste in the week before the MCO, regardless of the steady trend. Following the previous weekly pattern, a modest daily decrease in food waste was observed throughout the first week of the MCO. The MCO did not cause panic buying, which usually contributes to food waste due to over-buying, since the government guaranteed that the food supply was sufficient via the RM1 billion Food Security Fund (Prime Minister's Office of Malaysia, 2020).



**Figure 2.2:** Weekly Food Waste Collection in Selangor (19 February 2020–14 April 2020) (Latifah Abd Manaf, 2020).

### 2.1.7 The Influence of MCO to Food Waste Generation in Municipal Areas of Selangor

Figure 2.3 depicts the impact of MCO on food waste production in eight Selangor municipal districts. Tukey's post-hoc test revealed a statistically significant difference ( $p=0.05$ ) between food waste production and local authorities in Selangor municipal regions, as shown by the various letters in the Figure 2.3. Food waste production is also parallel during the first four weeks before the MCO, regardless of local authorities. Indeed, with cities projected to house 70% of the world population by 2050, food waste management is important in affecting urban quality of life (Liu, C, 2020). Before policymakers can make long-term choices regarding this problem, the definition of urban food waste must be investigated.



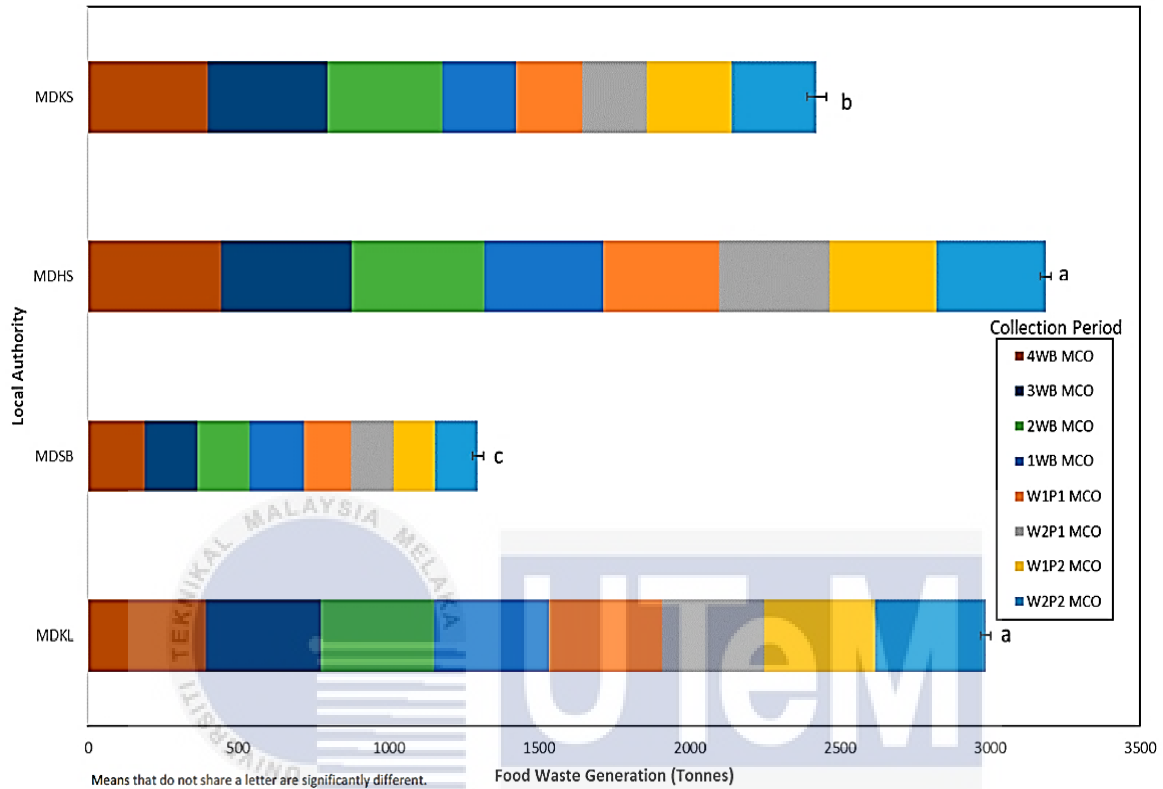
**Figure 2.3:** Weekly food waste collection contrast between municipal areas in Selangor before and during the MCO (Latifah Abd Manaf, 2020).



### 2.1.8 Impact of MCO on food waste generation in Selangor District Areas

The comparison of the tonnage of weekly food waste generated before and during the MCO according to the local authorities in the rural region of Selangor can be seen in the different letters in Figure 2.4. It is understood that food waste processing was constant for four weeks previous to the MCO. MDKL, MDSB, MDHS, and MDKS reported poor food waste collection 1 week before the MCO at 382.73, 181.03, 394.80, and 245.49 tonnes, respectively. The Selangor district area's low average quantity of daily food waste may be directly contrasted to 301,01 tonnes in rural Tunisia. The respondents had a positive attitude about food waste and indicated that the COVID-19 lockout impacted the waste rate of 93 percent of respondents and 80 percent of food purchasing decisions. In reality, the lockout

has improved food purchasing efficiency, which has led to a beneficial change in food waste behaviour (Jribi, S, 2020).



**Figure 2.4:** Weekly collection of food waste from the Selangor district areas (Latifah Abd Manaf, 2020).

Food waste collection has recorded significant discoveries as a consequence of the MCO's implementation in Selangor, which will offer evidence-based outcomes to advocate for stricter regulations throughout Malaysia. It was found that the MCO was able to decrease food waste output in both the municipal and district regions of Selangor throughout the limitation period. This is especially true in municipalities that had extremely high levels of food waste prior to the MCO. On the contrary, the majority of them reverted to the traditional food waste cycle for residents in district areas, feeding it to animals or using it as plant fertiliser. As a result, the minimal quantity of food waste before and during the MCO was clearly shown, with a very slightly smaller amount during the MCO.

Elks (2018) reported that the growing global population and changing lifestyles may increase food waste by almost a third in poorer nations by 2021. Household food waste is particularly significant since, in comparison to other levels, it accounts for the greatest portion of the supply chain and usually contributes to an increase in the percentage of urban trash (Think City, 2020). Food waste is expected to rise further as people and businesses recover from the disruption to the food supply system.

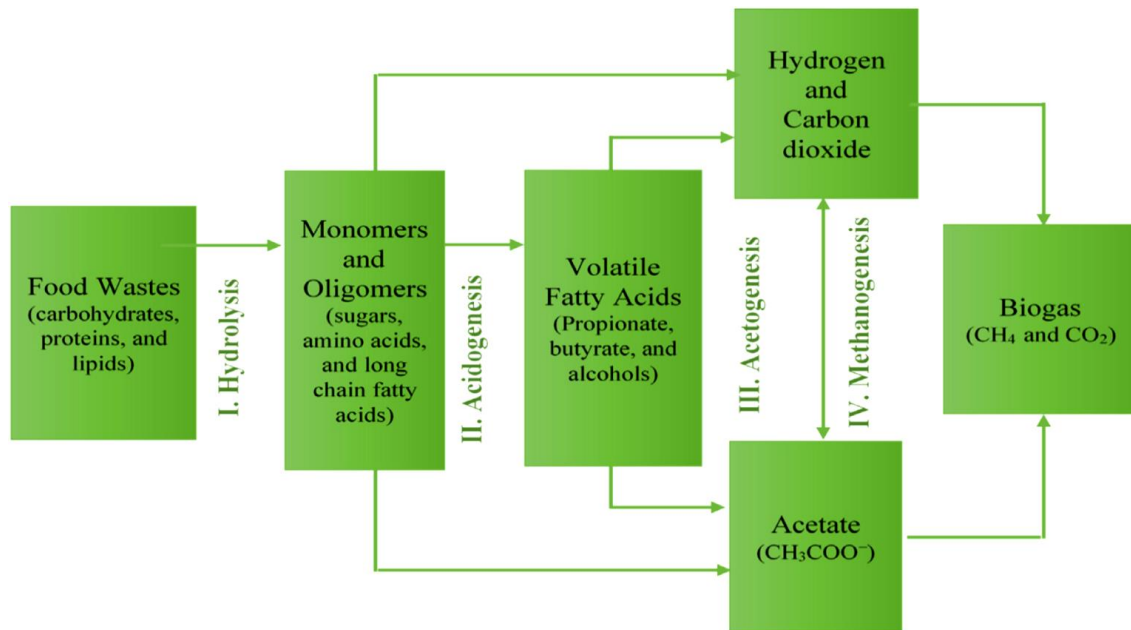
## **2.2 Anaerobic Digestion (AD)**

Anaerobic digestion (AD) is biological breakdown process of organic waste by anaerobic bacteria in the absence of oxygen via a series of processes such as hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Mir et al. 2016). Temperature, pH, C/N ratio, OLR, HRT, alkalinity, and volatile fatty acid concentration are some of the most significant factors affecting the effectiveness of an anaerobic digestion system. AD technology, as compared to fossil fuels, may reduce GHG emissions by utilising locally accessible sources. The main disadvantages of this method are the lengthy digesting retention time and poor heating value of produced gas (methane gas). The AD technique produces an alternative fuel in the form of biogas as well as a nutrient-rich fertiliser. As a result, it offers dual advantages in terms of fulfilling energy demand as well as trash management (Cuetos et al., 2008; Okuo et al., 2016; Saboor et al., 2017; Kapoor et al., 2019).

### **2.2.1 Important Stages of Anaerobic Digestion (AD)**

In the absence of oxygen, AD is the biological decomposition process of organic matter found in waste by anaerobic bacteria through a sequence of processes such as hydrolysis, acidogenesis, acetogenesis and methanogenesis (Mir et al. 2016). This can be systematically referred as in the following Figure 2.5. Each important stage was further detailed in the following section.





**Figure 2.5:** Production of biogas from food waste through the process of anaerobic digestion (AD) (Mir et al. 2016).

### 2.2.1.1. Hydrolysis

The goal of hydrolysis is to break down organic macromolecules into smaller components that may then be utilised by acidogenic bacteria. Extracellular enzymes have secreted by hydrolytic bacteria which may converted into sugar, long-chain fatty acids (LCFAs), and amino acids into carbohydrates, lipids, and proteins (Li, 2011). Hydrolysis has an optimal temperature of 30-50 ° C and at pH of 5-7 on its own, but there is no evidence of enhanced hydrolytic activity at below a pH of 7 (Azman, 2016)

### 2.2.1.2 Acidogenesis

Acidogenic bacteria may produce intermediate volatile fatty acids (VFAs) and other compounds by ingesting hydrolysis products via their cell membranes. Acidogenesis is often assumed to occur at a faster pace than all other phases of anaerobic digestion, with acidogenic bacteria having a recovery period of less than 36 hours (Deublein, 2008). Ammonia generation from deamination, which is thought to be an inhibitor of anaerobic

digestion at sufficiently high quantities, is a necessary result of amino acid breakdown (Park, 2014).

### **2.2.1.3 Acetogenesis**

Acetogenesis is the process of converting higher VFAs and other intermediates into acetate while also producing hydrogen (Hansen, 2013).

### **2.2.1.4 Methanogenesis**

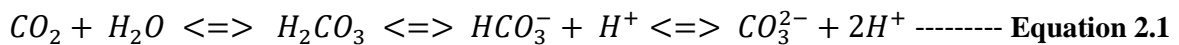
Methanogenesis is the last step of anaerobic digestion in which methanogenic bacteria consume useful intermediates to produce methane (Ferry, 2010). Methanogens establish syntrophic partnerships with 78 other microbes to generate methane from short-chain volatile fatty acids and alcohol produced from biodegradation of complex organic molecules, such as ethanol, propionate, butyrate, and so on (Shin et al., 2010; De Bok et al., 2004). In terms of environmental requirements of methanogenesis, methanogenic bacteria need higher pH than earlier stages of anaerobic digestion, as well as lower redox capacity, which has created significant difficulties for laboratory culture (Wolfe, 2011). At the same time, methanogens have somewhat slower regeneration period in anaerobic digestion than other microbes, ranging from 5 to 16 days (Deublein, D).

## **2.2.2 The Anaerobic Digestion Process Quantitative Tests**

The biological approach of AD is extremely complicated and is dependent on many variables, one of which is process parameters (Almasi et al., 2018). Numerous physical, chemical, and operational factors affect the process and efficiency of methanogenesis. The next section discusses how pH, temperature, hydraulic retention time (HRT), and substrate to inoculum ratio (S/I) influence the efficiency of anaerobic methane production.

### 2.2.2.1 The pH effect

The optimum pH for methanogenesis process is at the narrow range of 6.8 to 8.0. Methane production is significantly reduced at pH levels of less than 6.0 or higher than 8.5. The build-up of VFAs causes the pH to fall, while the accumulation of ammonia causes the pH to rise. The carbonic acid/bicarbonate/carbonate equilibrium (Eq. (2.1)) is a natural buffering mechanism that prevents excessively low pH levels.



The pH value of 6.5 bounces across this buffering system. Another buffering mechanism is equilibrium ammonia/ammonium (Eq. (2.2)), which avoids pH values that are too high.



However, the pH effect was not focused in this study due to reactor limitation as reactor has been placed in confined space.

### 2.2.2.2 Temperature effect

The AD technique is used at thermophilic (55-70 °C) or mesophilic (32-45 °C) temperature rates. Maintaining a consistent temperature in the digester is critical because methanogens, particularly thermophilic methanogens, are sensitive to temperature changes.

However, for some variables, mesophilic AD is still worth consideration, such as its better resistance to environmental fluctuations and faster rates of food waste solubilization at mesophilic temperatures (Zhang et al., 2014). Because of its high organic content, mesophilic AD is more stable than thermophilic AD for food waste. Guo et al. (2014) in their research, specified that the temperature is set at a mesophilic level of 37 °C (room temperature).

### 2.2.2.3 Effect of time of hydraulic retention (HRT)

Hydraulic retention time (HRT) refers to the mean amount of time in a digester for liquids to remain. HRT, which in literature also appears as  $\emptyset$ , can be determined as a digester length,  $V$ , and flow rate quotient of a digester,  $Q$ : The HRT can be define as in the following Equation 2.3.

$$\emptyset = \frac{V}{Q}$$

Equation 2.3

The retention time is likely the most important process element affecting methane production and rate of output (Gerardi, 2003). Increased retention time results in better reduction of volatile solids, increased digester capacity, and improved adaptation to pH fluctuations and hazardous chemicals. Mesophilic digestion is typically completed in 15-30 days (Mao, 2015). Reduced retention periods, on the other hand, result in a smaller digester capacity and, as a result, lower investment costs when processing biogas of the same quality and quantity (Chandra et al., 2012).

### 2.2.2.4 Ratio of substrate to inoculum effects (S/I)

The selection of an appropriate substrate to inoculum ratio (S/I) is critical for preventing volatile fatty acid (VFA) build-up during anaerobic digestion with the aim of improving methanogenic output. Microbial populations overburden the S/I, while a low S/I leads in high reactor volume needs and less CH<sub>4</sub> (Hinds et al., 2018). According to Hobbs et

al. (2018), S/I of food waste to anaerobic digested sludge at 0.3 came near to attaining CH<sub>4</sub> saturation by the conclusion of the test, but supplied the least quantity of CH<sub>4</sub> when compared to 1.0 and 2.1. Higher S/I of municipal trash may result in greater CH<sub>4</sub> production due to municipal waste buffering capability

### 2.2.2.5 Inoculums

The quality and amount of inoculum is key component in the AD process, affecting all four stages of diseases. The quantity of inoculum used affects the AD start-up phase (Motte et al., 2013). Among different animal inoculums, cow dung generates the most methane from food waste (Dhamodharan et al., 2015). According to reports, combining multiple inoculums would enhance biogas output (Gaur and Suthar, 2017). Table 2.5 summarised the different forms of inoculum used for the biogas production from food waste.

**Table 2.5:** The different forms of inoculum used for the production of biogas from food waste

| Inoculum   | Inoculum to substrate ratio (based on VS) | Max. CH <sub>4</sub> (Nm L/g VS) | Reference                  |
|--|---|----------------------------------|----------------------------|
| Livestock dung                                   | 2   | 227                              | Dhamodharan et al. (2015)  |
| Sludge from wastewater treatment plant           | 0.5                                       | 160                              | Kong et al. (2016)         |
| Inoculum from a digester fed with chicken manure | 0.25                                      | 580                              | Kong et al. (2016)         |
| Anaerobically digested food waste                | 2   | 522                              | Ebrahimi-Nik et al. (2018) |
| Sludge acclimated to food waste                  | 0.5                                       | 605                              | Kong et al. (2016)         |

### 2.2.3 Factor contributing to AD Device Failure

Anaerobic digestion is affected by numerous operational and environmental factors, which also correlate with the kinetics of different processes and biogas generation. Numerous physical, chemical, and operational factors affect the process and efficiency of methanogenesis. The next section elaborates on the key operating parameters affecting the efficiency of anaerobic methane production, such as temperature, pH, redox potential, the availability of critical micronutrients, organic loading rate (OLR), and hydraulic (HRT) (Yen and Brune, 2007). Table 2.6 summarised the factor contributing to process failure in AD system

**Table 2.6:** Factor contributing to Process Failure in AD System

| FACTOR  | JUSTIFICATION   | REFERENCE   |
|---|---|---|
| Temperature   | i. Temperature decrease can have drastic repercussions on a process operation<br>ii. Effect by organic loading rates (OLR) and hydraulic retention time (HRT) | <ul style="list-style-type: none"> <li>• (Oliveira et al., 2014).</li> <li>• (Yen and Brune, 2007)</li> </ul> |
| pH in digester tank                                 | Influences on biogas composition  | (Santos- Ballardo et al., 2015).  |
| Oxidation-reduction potential (ORP)                 | Influences the anaerobic digestion systems  | (Colmenarejo et al., 2004)  |
| Hydraulic retention time and substrate loading rate | The time required for microbes to remain in contact with organic matter.  | Alzate et al. (2012),   |
| Nutrients   | For their better growth, microorganisms need carbon, nitrogen, and phosphorus in the right concentration.   | (Slade and Bauen, 2013).  |

#### 2.2.4 Co-digestion

Anaerobic digestion of a single highly biodegradable organic substrate may result in process failure in the absence of any buffering agent for pH correction and appropriate external nutrient supply (Demirel and Scherer, 2008). This problem may be addressed by using another waste as a co-substrate, reducing the requirement for extra nutrients or alkali for pH control (Bouallagui et al., 2009). Co-digestion is the process of digesting several substrates at the same time. In recent years, researchers' interest in anaerobic co-digestion investigations has grown. It boosts biogas output by adding nutrients and controlling pH, which boosts methanogen synthesis.

#### 2.3 Biogas Generation

Biogas is being explored as a potential substitute for natural gas derived from fossil fuels (Morero et al., 2015). The generated biogas may be turned into heat and energy on-site (Boulamanti et al., 2013) or processed for injection of biomethane into the natural gas network for use as transportation fuel (Agostini et al., 2015) or household activities (Agostini et al., 2015; Russo and von Blottnitz, 2017). According to Browne and Murphy, the range of biogas production from food waste is 314 to 529 L CH<sub>4</sub> kg VS (-1) added, depending to the kind of reactor (continuous or batch reactor) and the source of food waste (Residential and business food waste, 2013) According to Mao et al. (2015), the major future trend is AD biogas optimization, which may be achieved by combining the factors affecting process efficiency (i.e. temperature regime, pH, C/N ratio, OLR, HRT) with the accelerators (i.e. selected biomass, inorganic additives). The biogas produced by anaerobic digestion is composed of methane (55-65 %) and carbon dioxide (30-35 %), as well as tiny amounts of other gases, including hydrogen sulphide, with concentrations ranging from several hundred to a few thousand ppm, water vapour, and other trace gases. According to Suzuki et al. (2012), biogas generated is of sufficient high quality to be utilised in internal combustion engines

when the methane content is at least 60%. Figure 2.6 depicts the biogas generation cycle (G, Northrup .2015).

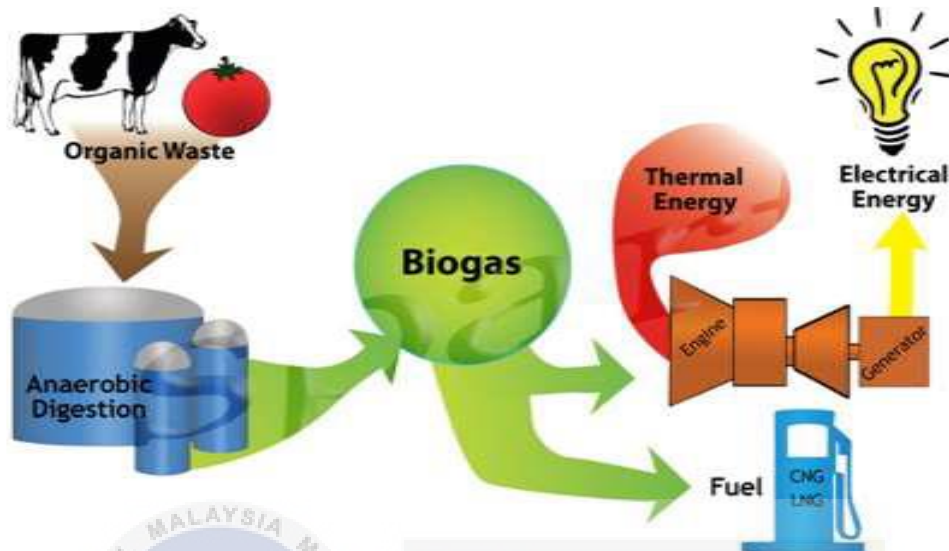
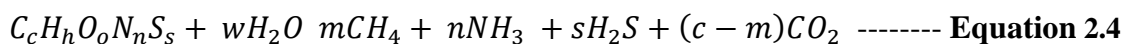


Figure 2.6: Biogas generation cycle (G, Northrup .2015)

### 2.3.1 Biochemical Reactions for Producing Biogas

Biogas is mostly composed of tiny amounts of hydrogen (H<sub>2</sub>), nitrogen (N<sub>2</sub>), hydrogen sulphide (H<sub>2</sub>S), oxygen (O<sub>2</sub>), water (H<sub>2</sub>O), and saturated hydrocarbons (methane (CH<sub>4</sub>) and carbon dioxide (CO<sub>2</sub>) (i.e., methane, propane). Table 2.7 discusses the whole composition of biogas. Under anaerobic circumstances, biogas is produced by a series of complicated biochemical processes that occur in four stages: hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Ghodrat et al., 2018). The complete bioconversion reaction of food waste into biogas is shown in Equation 2.4 (Deublein and Steinhauser, 2011):





Where,  $m = 1/8 (4c + h - 2o - 3n - 2s)$  and  $w = 1/4 (4c - h - 2o + 3n + 3s)$

The degradable food waste fraction consists primarily of carbohydrates (C-6 H-12 O-6), proteins (C-13 H-25 O-7 N-3 S), and lipids (C-12 H-24 O-6).

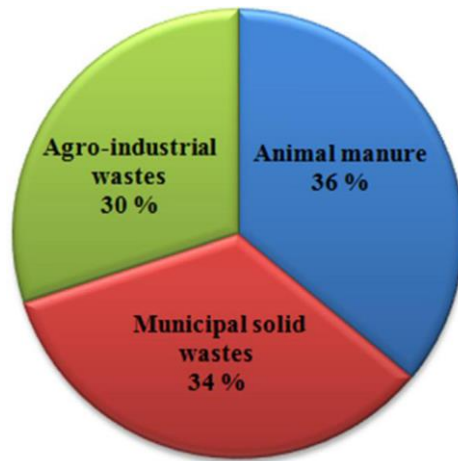
**Table 2.7:** Composition of biogas (Baciocchi Renato, 2013)

| Constituent       | Formula          | Concentration (v/v) | Combustible / Non- combustible |
|-------------------|------------------|---------------------|--------------------------------|
| Methane           | CH <sub>4</sub>  | 40–75%              | Combustible                    |
| Carbon dioxide    | CO <sub>2</sub>  | 15–60%              | Non-combustible                |
| Moisture          | H <sub>2</sub> O | 1–5%                | Non-combustible                |
| Nitrogen          | N <sub>2</sub>   | 0–5%                | Non-combustible                |
| Hydrogen          | H <sub>2</sub>   | Traces              | Combustible                    |
| Hydrogen sulphide | H <sub>2</sub> S | 0–5000 ppm          | Combustible                    |
| Oxygen            | O <sub>2</sub>   | < 2%                | Non-combustible                |
| Trace gases       | -                | < 2%                | -                              |
| Ammonia           | -                | 0–500 ppm           | -                              |

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### 2.3.2 Characteristic of Feedstock

Using AD technology, a large variety of waste types can be used as substrates for biogas production. A significant volume of lignocellulose waste from agricultural, municipal and other activities is obtained. Animal manure and slurry, sewage sludge, urban solid waste and food waste are the most common sources of waste used in the European energy industry (Soheil.A, 2017). Figure 2.7 depicts the total contribution to the production of biogas from the primary source of organic waste (Soheil.A, 2017).



**Figure 2.7:** The total contribution to the production of biogas from the primary source of organic waste (Soheil.A, 2017).

**Table 2.8:** Comparison of biogas yield and electricity generated from various substrates of potential. (Stucki M, 2011)

| Type                     | Biogas yield per ton           | Electricity produced per ton |
|--------------------------|--------------------------------|------------------------------|
|                          | fresh matter (m <sup>3</sup> ) | fresh matter (kW·h)          |
| Cattle Dung              | 55-68                          | 122.5                        |
| Chicken litter/dung      | 126                            | 257.3                        |
| Fat                      | 826-1200                       | 1687.4                       |
| Food waste (disinfected) | 110                            | 224.6                        |
| Fruit wastes             | 74                             | 151.6                        |
| Horse manure             | 56                             | 114.3                        |
| Maize silage             | 200/220                        | 409.6                        |
| Municipal solid waste    | 101.5                          | 207.2                        |
| Pig slurry               | 11-25                          | 23.5                         |
| Sewage slurry            | 47                             | 96.0                         |

The Malaysian Ministry of Agriculture and Agro-Based Industry, in collaboration with the Department of Veterinary Services (DVS), gathered preliminary data to estimate the potential for biogas production from farm animal waste. The statistics includes the livestock population maintained on farms in various areas of Malaysia, as well as the number of farm animals in Malaysian slaughterhouses in 2012. The quantity of animal excrement was then calculated based on body weight each year. The quantity of animal waste produced each year was used to predict the biogas production. It referred to the characteristics that were directly related to the measurement of biogas. The quantity of methane produced by various chemical components of the same fuel varies. Fats and proteins generate more methane than carbs, and lignin is not biodegradable in anaerobic digestion (AD).

### 2.3.3 Population of Livestock

Malaysia is a tropical country in Southeast Asia that consists of Peninsula Malaysia (West Malaysia) and East Malaysia (Sabah and Sarawak). Malaysia has an equatorial climate with 200-250 cm of rain each year, making it an important agricultural and animal producing region. According to DVS, the Ministry of Agriculture and Agro-Based Industry, Malaysia has 118,674 buffalo, 742,558 cattle, 458,646 goats, and 131,923 sheep in 2012.

**Table 2.9:** Malaysian Livestock Population in 2012 (Federation of Malaysian Livestock Farmers' Association (FLFAM)).

| Region              | Buffalo | Cattle  | Goat    | Sheep   | Poultry     |
|---------------------|---------|---------|---------|---------|-------------|
| Peninsular Malaysia | 65,858  | 663,563 | 394,905 | 127,671 | 703,310,511 |
| Sabah               | 45,539  | 63,875  | 49,146  | 2070    | 45,738,500  |
| Sarawak             | 7277    | 15,120  | 14,595  | 2182    | 17,266,000  |

### 2.3.4 Biogas Production Capacity

In particular, livestock waste has been established as the potential feed stock for sustainable biogas generation in the AD process (Than TMM. 2005). The potential for processing biogas from animal and poultry waste is shown in Table 2.10.

**Table 2.10:** Impact on Process Efficiency of Co-Digestion of Protein-Rich Biomass with Carbon-Source Additives.

| Protein-rich waste biomass         | Carbon-source additive  | Results  | References              |
|------------------------------------|---|--|-------------------------|
| Cow manure                         | Crop silage (70% VS)  | 109% improvement in CH <sub>4</sub>  | (Comino E, 2010)        |
| Manure and slaughterhouse residues | Crops   | 43% improvement in CH <sub>4</sub> yield   | (Díaz JP, 2011)         |
| Slaughter house residue            | Crops and food waste (1:1:1 ratio)                                | 200 percent increase in OLR, healthy functioning and fair bacterial representation                       | (Pagés-Díaz J, 2017)    |
| Pig slaughter house residue        | Tomato industry waste (4:1 ratio)                                 | 80% reduction in COD and improvement in biogas generation  | (González A, 2013)      |
| Cow manure                         | Thermally pre-treated food waste at 121 °C and 30 min with 30% TS | Improvement in biogas output of 70-85 percent and 62-81 percent improvement in producing CH <sub>4</sub> | (Arelli V, 2018.)       |
| Chicken manure mixing with         | Corn straw (1:3 ratio)  | 600% improvement in CH <sub>4</sub> generation   | (Feng J, 2017)          |
| Livestock manure                   | Cabbage waste with 1:1 ratio                                      | Significant improvement in methane generation  | (Gaibor-Chávez J, 2018) |
| Dairy manure                       | Spent mushroom substrate (1:3 ratio)                              | 400% improvement in biogas yield   | (Luo X, 2018)           |
| Pig manure                         | Corn stover and cucumber residue (5:2:3 ratio)                    | 350% improvement in CH <sub>4</sub> generation   | (Wang Y, 2018)          |

### 2.3.5 Biohydrogen

Biohydrogen is a kind of biofuel similar to bioethanol, biodiesel, and bio-oil. Both chemical and biological processes may be used to produce hydrogen. As a result, biohydrogen refers to a technique of producing hydrogen biologically (through microorganisms) in a bioreactor. In simple words, biohydrogen is the biological conversion of hydrogen into biohydrogen by microorganisms (Hallenbeck Pc, 2002). This section would briefly discuss about the pathways for producing biohydrogen and the factors that affecting the production of biohydrogen.

#### 2.3.5.1 Pathways for producing biohydrogen

There are several pathways for producing biohydrogen. For instance, through photolysis process, photo-fermentation process and dark fermentation process (Hallenbeck Pc, 2002).

##### i. **Photolysis**

Through the photosynthetic capacity of algae and cyanobacteria, hydrogen may be generated directly through a water-splitting process. Biohydrogen is produced via direct light absorption and electron transfer to hydrogenases and/or nitrogenases enzymes. Microorganisms release extra electrons in anaerobic or high-energy circumstances by using the hydrogenase enzyme, which transforms hydrogen ions to hydrogen gas (Turner et al., 2008).

##### ii. **Photo-fermentation**

Unlike the photolysis process, in which hydrogen is generated directly or indirectly from water by cyanobacteria and/or green algae, purple photosynthetic microorganisms may generate hydrogen from organic substrates through photo-fermentation. Despite comparatively lower hydrogen yields from photosynthetic bacteria, the fermentative route

is a potential biohydrogen generation method, owing to its faster rate of hydrogen evolution in the absence of any light source (Wang et al., 2010a). The method is adaptable in terms of the microorganisms' diet (Redwood et al., 2009).

### **iii. Dark fermentation**

Among the bioproduction methods, dark fermentation under anaerobic circumstances seems to be the most advantageous. Using different organic substrates and wastewaters, fermentation may be carried out at greater rates and at a reduced cost (Hallenbeck and Ghosh, 2009). On carbohydrate-rich substrates produced without the requirement for light energy, dark fermentation utilises mainly anaerobic bacteria, but certain algae are also utilised (Kapdan and Kargi, 2006).

#### **2.3.5.2 Factors affecting biohydrogen production**

There are several factors that affecting the production of biohydrogen. For instance, the parameters such as hydraulic retention time (HRT), nutrients, temperature, substrate concentration and feedstock (Kuan-Yeow Show, 2011). The following section provides details for each parameter.

##### **i. Hydraulic retention time (HRT)**

Hydraulic retention time (HRT) may be used to identify microbial communities whose growth rates can keep up with the mechanical dilution caused by continuous volumetric flow. Zhang et al. (2006a) discovered that reducing the HRT from eight to six hours reduced microbial diversity associated with propionate inhibition without altering the presence of dominating species, resulting in an increase in hydrogen output. Other studies have found similar findings (Hussy et al., 2003). These findings demonstrated that hydrogen yield, which is a function of microbial populations, may be influenced by HRT (Zhang et al., 2006a).

## **ii. Nutrients**

Nitrogen, phosphate, and other inorganic trace minerals are required supplements for carbohydrate-based feedstocks in hydrogen fermentation processes in order to achieve optimum cell growth and hydrogen generation. Previous research has shown that organic nitrogen seems to be more suitable for hydrogen evolution than inorganic nitrogen (Yokoi et al., 2001).

## **iii. Temperature**

Microbes may produce hydrogen at temperatures ranging from 15 to 85 degrees Celsius (Kanai et al., 2005), however from the laboratory-scale research has found that about 73% of studies used mesophilic cultures (Li and Fang, 2007). Chang and Lin (2004) had investigated the hydrogen production capability of a mixed culture at temperatures ranging from 15 to 34 degrees Celsius and discovered that hydrogen yield and specific hydrogen production rate increased with temperature, reaching maximum values of 359 mmol H<sub>2</sub> l<sup>-1</sup> d<sup>-1</sup> and 1.42 mol H<sub>2</sub> mol<sup>-1</sup> glucose at 30–34 degrees Celsius and 28–32 degrees Celsius, respectively.

## **iv. Substrate concentration**

The impact of substrate concentration on hydrogen generation, on the other hand, has been a source of contention. Kim et al. (2006) discovered that hydrogen yield increased with increasing glucose concentration from 10 to 35 g l<sup>-1</sup> at an HRT of 12 h, whereas Kyazze et al. (2006) investigated continuous hydrogen production at 12 h HRT on 10–50 g l<sup>-1</sup> sucrose and discovered that hydrogen yield decreased from 1.7 ± 0.2 mol H<sub>2</sub> mol<sup>-1</sup> hexose added at 10 g l<sup>-1</sup> sucrose.

## v. **Feedstock**

Because simple sugars like glucose, sucrose, and lactose are easily biodegradable, they are chosen as model substrates for hydrogen generation. However, the prices for pure carbohydrate sources are too expensive for large-scale hydrogen generation, which can only be economically feasible when it based on renewable and low-cost sources. Carbohydrates are the primary source of hydrogen. According to various investigations of biohydrogen fermentative processes, the wastes and biomass are high in sugars and/or complex carbohydrates. This could be the best feedstocks for biohydrogen production (Lo et al., 2008, 2009a; Luo et al., 2011; Ntaikou et al., 2010).

## **2.4 Reactor Design**

In this section, the parameters in reactor operation, application of biogas in household digester, disadvantages and related environmental and social aspects of biogas were discussed.

### **2.4.1 Parameters in Reactor Operation**

There are several parameters in the operation of biohydrogen reactor. For instance, material for construction, substrate consumption and biogas storage and maintenance of reactor (Karthik Rajendran, 2012).

#### **i. Materials for Construction**

Resources used in the building of home digesters are determined by geological, hydrological, and local factors, as well as locally accessible materials (Shian, 2019). Different materials with better characteristics and cheaper prices have been brought to the market in recent years as a result of technical advancements. With the advancement of technology, PVC and polyethylene were substituted since they are less expensive (Rodriguez, 2017). Table 2.11 summarises several building materials and their benefits and drawbacks.



**Table 2.11:** Building materials for biogas reactor

| Material                  | Modification   | Advantages                                 | Disadvantages                      | References   |
|---------------------------|--|--|------------------------------------|--------------|
| Poly vinyl chloride (PVC) | PVC red mud (combined with metal)                              | Less weight                                | Plastic has a short life lifetime. | Ferrer, 2011 |
| Neoprene and rubber       | Nylon reinforcement  | Elastic weather resistance                 | Expensive<br>Low tensile strength. | Kanwar, 2004 |
| Bamboo and wood supports  | Typically used as a support material and reinforced with flax. | Material that is easily accessible locally | It is readily broken.              | Gautam, 2009 |

**ii. Substrate Consumption**

The impact of substrate concentration on hydrogen generation, on the other hand, has been a source of contention. Kim et al. (2006) discovered that hydrogen yield increased with increasing glucose concentration from 10 to 35  $\text{gl}^{-1}$  at HRT of 12 hrs, whereas Kyazze et al. (2006) investigated continuous hydrogen production at 12 hrs HRT on 10–50  $\text{gl}^{-1}$  sucrose and discovered that hydrogen yield decreased from 1.7 to 0.2  $\text{mol H}_2 \text{ mol}^{-1}$  hexose added at 10  $\text{gl}^{-1}$  sucrose. When the CSTR feeding strength was raised from 10 g glucose to 40 g glucose, hydrogen output decreased at HRTs of 2.5 hrs and 10 hrs. (Van Ginkel and Logan, 2005). These investigations show that, in addition to substrate concentration, additional operational parameters such as HRT and microbial culture composition influence continuous hydrogen generation.

### iii. **Biogas Storage and Maintenance of Reactor**

Storing the generated biogas is often a significant issue. Biogas may be immediately delivered to the kitchen or kept in a pressurised tank, floating drum storage, gas cylinders, or tyre tube. Storing the biogas alleviates the issue of lower flow rate during cooking. Tyre tube may be used to transfer biogas from one location to another (Shian, 2002). A 'T' shaped valve may be used to relieve excess pressure in the storage container (Rodriguez, 2001).

The quantity of biogas generated in the digester is determined by the material supplied, material types, the C/N ratio, the digestion duration, and the temperature (Omer, 2003) For example, highly concentrated influent slows the fermentation process, while diluted influent promotes scum development. To maintain solids concentration, the quantity of water and biomass supplied should be proportionated (Zhang, 2010). Every day, the digester should be fed. However, free fermentable carbohydrates raise the concentration of volatile fatty acids, which inhibits methane-forming bacteria. After two months of operation with a consistent organic loading rate (OLR), the stable state of biogas generation is usually seen.

#### 2.4.2 Applications of Biogas in Household Digesters

There are several applications of biogas in household digesters. For instance, cooking and heating, fertilizer, lighting and power generation (Karthik Rajendran, 2012).

##### i. **Cooking and Heating**

The biogas generated by home digesters is mostly utilised for cooking (Gautam, 2009). Typically, the quantity of biogas utilised for cooking is between 30 and 45 m<sup>3</sup> per month. This may be compared to other frequently used fuels such as kerosene, which consumes between 15 and 20 L per month, and Liquefied Petroleum Gas (LPG), which consumes between 11 and 15 kg per month. The energy equivalents for biogas, kerosene, and LPG were about 300, 200, and 150 kWh, respectively (Gosling, 2003). The excess biogas produced by the home digester may be utilised to heat water and space (He, 2010).

## **ii. Fertilizer**

The digestate produced by the digester is higher in nitrogen, phosphorus, and potassium and may be utilised as fertiliser (Gautam, 2009). Digestate enhanced potato growth by 27.5% and forage cultivation by 1.5% as compared unadded additional fertiliser. These nutrient concentrations were readily absorbed by plants due to anaerobic digestion of organic waste (Garfi, 2011). The wastewater may be utilised directly as a fertiliser in agriculture. When exported, digestate has a significant economic value. The dried effluent may potentially be utilised as an adsorbent in industrial wastewater to remove lead (Yamuna, 2005). Biogas slurry may aid in the growth of algae, water hyacinth, duck weed, and fish poly-aquaculture.

## **iii. Lighting and Power Generation**

Another important use of home biogas is for lighting and electricity production. Biogas from digesters is transported to a combustion engine in many industrialised nations to be converted into electrical and mechanical energy (Gautam, 2009). To ignite biogas, a liquid fuel is required. Diesel fuel may also be mixed with biogas to generate electricity. In Pura, India, for example, a well-studied community biogas digester can power a modified diesel engine and an electric generator (Reddy, 2004). According to Bari (2013), utilising biogas as a fuel does not reduce engine performance by up to 40% carbon dioxide. Biogas may also be used to power motors when combined with gasoline or diesel, and it can aid in the pumping of water for irrigation (Lane, 2007).

### 2.4.3 Disadvantages

Despite many benefits of home biogas digesters, there are a few drawbacks to consider. Anaerobic digestion is a time-consuming process that requires a lengthy HRT of more than 30 days (Martins, 2009). This raises the digester's volume and expense. Other constraints in biogas production include low loading rates and delayed recovery after a failure. Another restriction is the year-round temperature variation. Because of the reduction in biogas output during the winter months, cold nations find it difficult to use this technology (Yadvika, 2006). People often quit using home digesters in the long-term owing to lack of understanding, gas leakage, delayed recovery, poor gas output, and an insufficient supply of substrate (Karthik Rajendran, 2012).

### 2.4.4 Environmental and Social Aspects of Biogas Digesters

Climate change is one of the most serious environmental issues confronting the world today. In the past, non-sustainable energy use has led into global warming, which must be carefully addressed (Bilen, 2008). Household digesters have the potential to alleviate environmental pressures by decreasing deforestation and greenhouse gas emissions, soil erosion, and loss of cultivable land (Gautam, 2009).

Greenhouse gases (GHG) are a significant contributor to global warming since they are released into atmosphere mostly through combustion of fossil fuels such as coal, oil, and natural gas. Rural biogas generation may help to mitigate global warming. The usage of biogas for rural families resulted in environmental, economic, and social advantages (Yang, 2011). Despite the fact that both methane and carbon dioxide are significant contributors to the greenhouse effect, methane has a 21-fold greater global warming potential than carbon dioxide. However, a study of homes equipped with and without biogas systems, including gas leakage in the biogas systems, showed that families with biogas plants emit 48% less than households without biogas systems. It's worth noting that just 10% of homes experienced methane leaks. According to studies, replacing firewood and coal with biogas reduces CO<sub>2</sub> and SO<sub>2</sub> emissions by 397–4193 thousand tonnes and 21.3–62.0 thousand tonnes, respectively (Wu, 2011).

If sludge from the biogas digesters is not utilised correctly, it creates a breeding ground for insects that transmit illnesses (Surindra, 2010). Biogas slurry has the potential to be a significant resource for earthworm cultivation. Slurry that has been combined with plant-rich materials is also a good substrate for vermicomposting (Suthar, 2009).

Cattle dung is often utilised as compost or dung cakes for cooking, which is neither sanitary nor cost-effective. Burning dung cakes not only causes pollution, but it also results in loss of useful fertiliser. However, if the dung cake is put straight to the field, it will result in a complete loss of fuel, in addition to pollution (Bala, 2002). Anaerobic digestion is a safe and profitable method of disposing of this cow manure.

## 2.5 Previous Study Related with gas yield Quantification

This section is briefly explained about previous study related with gas yield quantification. For instance, quantification of biohydrogen and quantification using gas chromatography (GC-TCD).

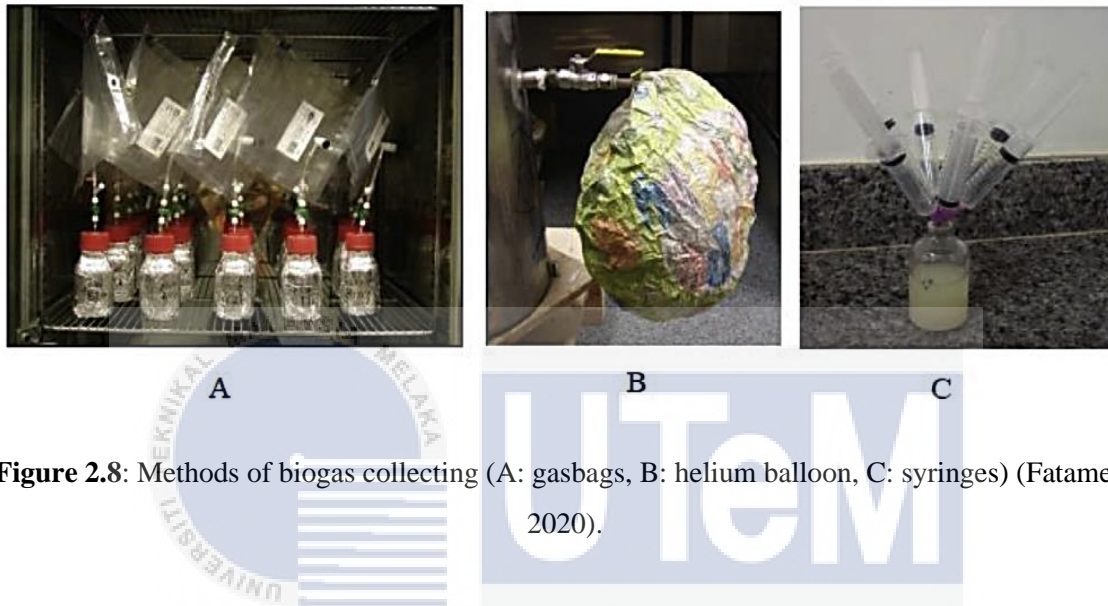
### 2.5.1 Quantification of Biohydrogen

There are several methods to quantify biohydrogen gases. For example, through gas chromatography (GC-TCD), commercial gas meter and hydrogen sensor instrument.

#### i. Gas chromatography

Gas chromatography is an essential separation technique in which the components of sample partition between two phases of stationary and mobile phases, and it was used to evaluate qualitative and quantitative substances that can be vaporised without decomposition (Gordon, 2000). Gas chromatography can examine solid, liquid, and gas samples (Ahuja, 2003). As an analytical tool, gas chromatography may be used to examine and quantify gases such as methane (CH<sub>4</sub>), carbon dioxide (CO<sub>2</sub>), hydrogen (H<sub>2</sub>), and others. The advantage of GC over other techniques of gas measurement is its ability to quantify both quantitative and

qualitative biogas generated (Prakash, 2011). This technique is extremely helpful for measuring biohydrogen since it was simple to use, precise, and sensitive, but it needs special equipment and setups. The drawbacks of GC are that it is more costly and has a bigger footprint than other techniques of measuring biogas (Hubert, 2011). Figure 2.8 depicts the methods of biogas collecting (A: gasbags, B: helium balloon, C: syringes) (Fatameh, 2020).



**Figure 2.8:** Methods of biogas collecting (A: gasbags, B: helium balloon, C: syringes) (Fatameh, 2020).

ii. **Commercial gas meter**

The diffusion of biogas in barrier solutions is a drawback of water displacement technique, which is addressed by the specified gas meter. A gas meter is a basic, easy-to-use device for measuring gases that is accurate, repeatable, and durable (Prakash, 2011), and it was used to monitor the flow of hydrogen gas. Gas meters have the potential to be utilised in large-scale commercial applications (Walker, 2009). The ideal gas meter must be inexpensive, accurate, capable of measuring a broad flow range, capable of operating at low gauge pressures, capable of datalogging, need minimal maintenance, and cause negligible pressure fluctuations (Smith, 2008). The gas meter is divided into two types: dry and wet, as well as commercial and experimental applications. The functioning of a dry and wet gas meter is identical, except with a dry gas metre, the water seal is replaced by a sliding valve seal (Baker, 2001). Wet gas meters are cumbersome. Wet gas meters are highly accurate under ideal circumstances, especially at low flow rates.

Dry gas meters are more handy than wet gas meters, although they are less accurate. Biogas output in laboratory studies is typically modest, often less than 5 mL/min, and conventional gas flow meters are not usually appropriate for measuring tiny quantities of biogas with low flow rates. Gas flow meters were created and developed to address this issue. The gas meters were designed with an adjustable resolution meter, low back-pressure, and a broad flow rate capability. For biohydrogen measurement, both wet and dry commercial gas meters were used. The dry-type gas meter, on the other hand, was more often utilised than the wet-type gas meter. Beckers (2015) utilised two digital flow gas meters in series to monitor the continuous biohydrogen, and the findings were validated using the flow meters.

*Clostridium butyricum* CWBI1009 and glucose monohydrate as the substrate were used in hydrogen generation tests in a 2.5 L AnSBR. The hydrogen proportion in biogas was then determined using a GC outfitted with a TCD and CarboPLOT P7 column and nitrogen as the carrier gas. Amorim studied the amount of hydrogen produced by dark fermentation of glucose in an anaerobic fluidized bed bioreactor with expanded clay as support (2009). They utilised a gas metre followed by GC-TCD to determine the rate and percentage of hydrogen gas generated. A wet gas metre was used to record the amount of biohydrogen generated by anaerobic digested sludge on a daily basis. The gas was characterised using a GC equipped with TCD and columns (Lay.J, 2000).

### iii. Hydrogen sensor

A sensor is an electronic device that measures and monitors environmental changes, converts those changes to electrical signals, and transmits that information to other electronic instruments for computation and analysis (Pandey, 2019). When compared to traditional techniques of hydrogen detection, hydrogen sensors are transducer devices that detect hydrogen gas. They have benefits such as cheaper cost, smaller size, and faster reaction. Catalytic, mechanical, optical, electrochemical, metal oxide, thermal conductivity, and other kinds of hydrogen sensors exist (Hubert, 2011). In terms of performance, each kind of hydrogen sensor has benefits and drawbacks. Sensor types' performance characteristics include measurement range, sensitivity, selectivity, stability, accuracy, and reaction time. Sensor performance may be limited by poor selectivity, excessive power consumption, being

poisoned by other gases or materials, having a short lifespan, and having a sluggish reaction time. A good sensor must be cheap in cost, low in maintenance, have a quick reaction time, be simple to install and operate, and be accurate. A sensor is also chosen based on the ambient working circumstances, detection needs, and sensor performance capabilities (Gu, 2011). The measuring sensor should not interfere with the generation of biogas. There are many kinds of hydrogen sensors on the market, each based on a different method for detecting hydrogen. Hydrogen sensors are used in applications such as hydrogen generation, storage, transit, and consumption. Because hydrogen is colourless, odourless, tasteless, and explosive, industry often utilised hydrogen sensors to detect hydrogen leaks. Hydrogen sensors are used in a variety of sectors, including petroleum, petrochemical, medical diagnostics, fuel cells, nuclear power plants, and so on. The goal for developing hydrogen sensors is to enhance performance while lowering prices (Najjar, 2019).

### **2.5.2. Quantification using Gas Chromatography - Thermal Conductivity Detector (GC-TCD)**

Biogas is a gas that includes methane, carbon dioxide, hydrogen, and hydrogen sulphide, with the major components being methane and carbon dioxide. The components of biogas produced (methane, hydrogen, and carbon dioxide) may be analysed using GC, which is a powerful analytical technique (Andersen et al. 2010; Kolb 2006). TCD is less sensitive than FID, but it is more widely employed to identify light substances (Poole 2003). In the investigation of biogas in GC, a packed column and a TCD detector are employed. Nitrogen is utilised as the carrier gas at a flow rate of 50 mL/min. The column, injector, and detector have temperatures of 40, 100, and 150°C, respectively. To inject biogas into the GC, a pressure-lock syringe is utilised.

Based on gas volume versus peak area, a calibration curve is generated for each gas. The calibration curve is used to determine the gas volume and percentage of gas composition. Biogas samples are collected in the ambient pressure mode at the start of each period (the pressure in the digester is released by inserting a needle in the septum while the other end of the tube connected to the needle is placed in a water container to avoid air introduction into



the digester). At the conclusion of each period, samples are collected in high pressure mode. The samples are GC-analysed, and the total amount of biogas produced is calculated using the following equations (Equations 2.5 and 2.6):

$$\text{Gas volume in the digester (ml)} = \frac{\text{Syringe volume } (\mu\text{L})}{\text{Sample volume } (\mu\text{L}) \times \text{Free volume of the digester (mL)}}$$

----- **Equation 2.5**

Produced gas volume during one interval = gas volume in the digester at high pressure (end of interval) - gas volume in the digester at environmental pressure (beginning of interval)

-----**Equation 2.6**

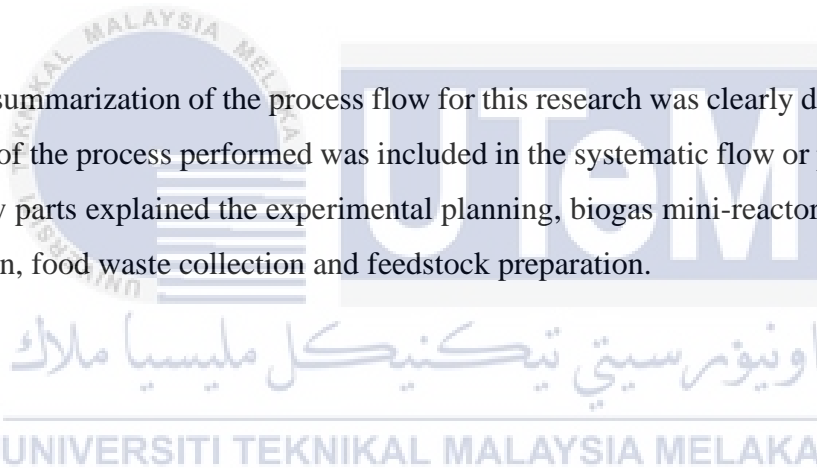
For research of produced biogas from biomass, the volume of generated biogas from control sample (inoculum and water) should be derived from the quantity of produced biogas from the sample. To verify that the inoculum is active, a control sample of pure cellulose or Avicel may be utilised.

UNIVERSITI TEKNIKAL MALAYSIA MELAKA

## CHAPTER 3

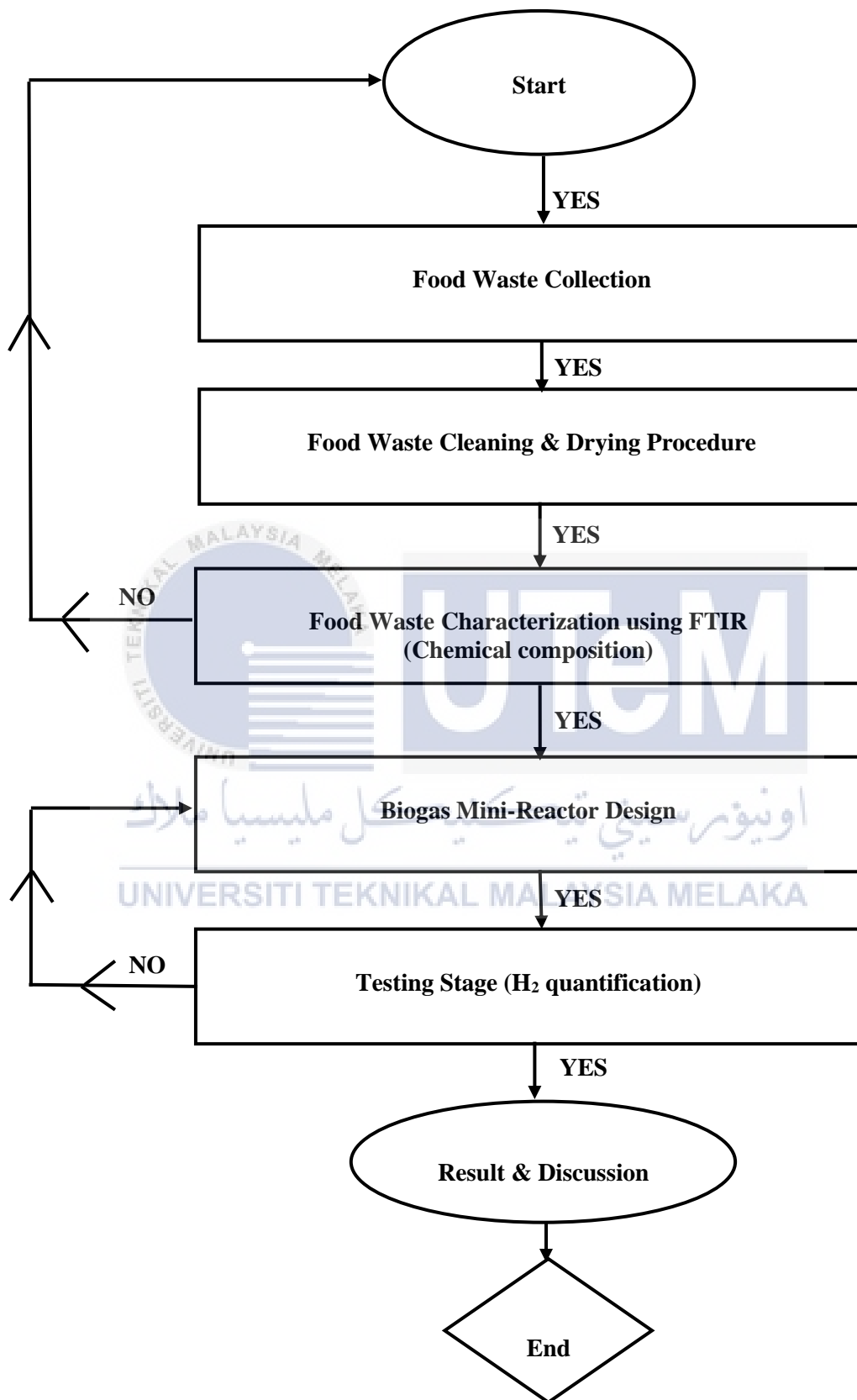
### METHODOLOGY

The summarization of the process flow for this research was clearly described in this chapter. All of the process performed was included in the systematic flow or procedure. The methodology parts explained the experimental planning, biogas mini-reactor design, output quantification, food waste collection and feedstock preparation.



#### 3.1 Introduction

Figure 3.1 depicts the flow chart of methodology for this research which consists of preparation and the experimentation works. At beginning of the flow, the research was started with food waste collection in one of popular fast-food outlet located at Gangsa, Melaka. After that, the feedstock was undergoing the preparation by cleaning and drying method. Next, the food waste was undergone the characterization to determine the chemical composition through Fourier Transform Infrared (FTIR) analysis. Next, the design and fabrication of biogas reactor tank will be carried out. After that, the testing stage for biohydrogen generation was implemented. Lastly, the conclusion was made based from the analysis.



**Figure 3.1:** Flow chart of the overall methodology

## 3.2 Food Waste Collection and Feedstock Preparation

This study has narrowed down the food waste collection area, was at one of popular fast-food outlet located in Gangsa, Melaka. Methods of food waste collection and feedstock preparation was discussed at the following section. The analyse of food waste in this study is conducted by using the Fourier Transform Infrared Spectroscopy (FTIR) analysis only.

### 3.2.1 Food Waste Collection and Planning

The major ingredients of food wastes were chicken leftover and rice leftover. The chicken leftover was obtained from one popular fast-food outlet in Gangsa, Melaka, for two times per week. The method of collecting chicken leftover is through walk in method. For first time collection, the fast-food outlet worker was helping to sort out the food waste (chicken leftover, potato waste and coleslaw waste) (Figure 3.2) while for the second time, the fast-food outlet was preferred to give me the garbage bag that include multiple waste (Figure 3.3).



**Figure 3.2:** A fast food outlet worker was sort out the food waste (chicken leftover, bun leftover, potato leftover and coleslaw leftover) from garbage bag for the first day of collection.



**Figure 3.3:** Chicken leftover (Meat and bones) were sorted out from the garbage bag.

After two times of collection, the chicken leftover was sorted out and bone and meat were separated accordingly as shown in Figure 3.4. After that, the separated meat from chicken leftover were rinsed until it was clean and shake well afterwards (Figure 3.5).



**Figure 3.4:** Chicken leftover (Bones and meats) were separated accordingly to two different medium.



**Figure 3.5:** Meats from chicken leftover were rinsed with tap water and shake well after rinsed.

Until that, meats from chicken leftover were dried in the oven with 150°C, 40 minutes for two times as shown in Figure 3.6. Then, the chicken meats were left to dried under the sun until it was completely dried ( Figure 3.7).



**Figure 3.6:** Chicken meats were dried for 150°C, 40 minutes for two times in the oven.



**Figure 3.7:** Chicken meats is then dried under the sun until it was completely dried.

After that, dried chicken meat was grinded in the blender until it was becoming powder form as shown in Figure 3.8.



**Figure 3.8:** Dried chicken meat in the powder form.

Dried chicken powder and tap water was weighed into 369 grams (Figure 3.9) and was added together to become 738 grams as shown in Figure 3.10.



**Figure 3.9:** A 369 grams of dried chicken powder and tap water respectively.



**Figure 3.10:** A 738 grams of dried chicken powder and tap water.



Then, 738 grams of dried chicken powder and tap water was divided into four and get 184.5 grams to meet the requirements of substrate ratio (Figure 3.11).



**Figure 3.11:** A 184.5 grams of dried chicken powder and tap water.

On the other hand, rice leftover that have collected from household (Figure 3.12) was also dried in the oven to get a completely dried rice form as shown in Figure 3.13.



**Figure 3.12:** Rice leftover that collected from household.



**Figure 3.13:** Dried rice leftover that have dried completely from oven.

Then, dried rice leftover have been blended by using blender (Figure 3.14). After several minutes, dried rice leftover has turned into rice powder form as shown in Figure 3.15.

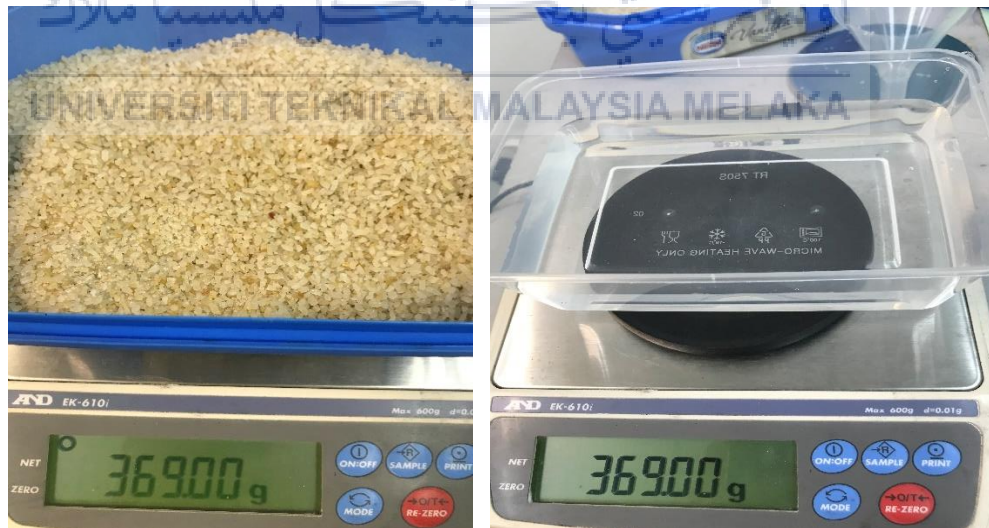


**Figure 3.14:** Dried rice leftover was blended by using a blender.



**Figure 3.15:** Rice powder form.

Rice powder and tap water was weighed into 369 grams (Figure 3.16) and added together to become 738 grams as shown in Figure 3.17.

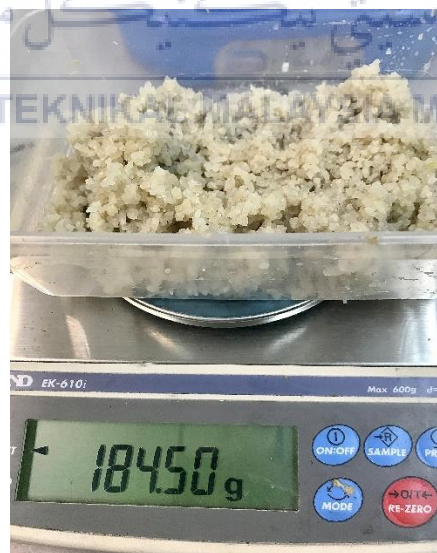


**Figure 3.16:** A 369 grams of rice powder and tap water respectively.



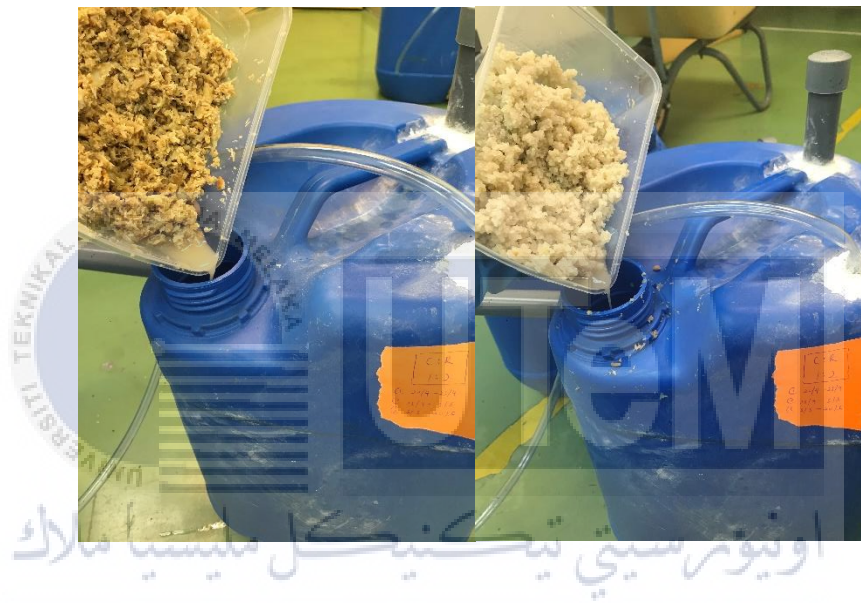
**Figure 3.17:** A 738 grams of rice powder and tap water.

After that, 738 grams of rice powder and tap water was divided into four and get 184.5 grams to meet the requirements of ratio (Figure 3.18).



**Figure 3.18:** A 184.5 grams of rice powder and tap water.

There are three ratios of chicken to rice in this experiment, it was 2:1, 1:1 and 1:2 respectively. Ratio of 1 indicate 184.5 grams and ratio of 2 indicates 369 grams. For instance, 2:1 is equal to 369grams to 184.5 grams. Therefore, after substrate (chicken leftover and rice leftover) was weighed, the substrate was inserted into three digesters accordingly as shown in Figure 3.19.



**Figure 3.19:** Substrate ( Chicken leftover and rice leftover) was poured into the reactor accordingly.

For inoculum (cow dung) that have been collected from a cow farm at Bernam Ulu, Melaka. Figure 3.20 shows the fresh cow dung that was collected by using a shovel.



**Figure 3.20:** Fresh cow dung that have collected by using a shovel.

Fresh cow dung was then collected into seven litres of plastic bucket as shown in Figure 3.21.



**Figure 3.21:** Plastic bucket that contain seven litres of fresh cow dung.

Fresh cow dung was then left to fermented into ten days to enhance the bacterial activity. After ten days, fermented cow dung was observed to be appeared in army green colour as shown in Figure 3.22.



**Figure 3.22:** Fermented cow dung that appeared army green in colour.

Next, seven litres of tap water were added into seven litres of fermented cow dung to meet the ratio of 1:1 (Figure 3.23). After that, seven litres of tap water and seven litres of fermented cow dung were stirred together by using a steel stick as shown in Figure 3.24.



**Figure 3.23:** A seven litres of tap water were added into seven litres of fermented cow dung.



**Figure 3.24:** A seven litres of tap water and seven litres of fermented cow dung were stirred by using a steel stick.

After stirring, cow dung slurry was formed as shown in Figure 3.25. A seven litres of tap water have mixed with seven litres of fermented cow dung to produce 14 litres of cow dung slurry. It was then divided into three to meet the ratio requirements and become 4.67 litres which was also 4670 grams.



**Figure 3.25:** A 14 litres of cow dung slurry.



Then, 4670 grams of cow dung slurry is then divided into 500 grams that marked by the orange strip as shown in Figure 3.26. Then, 500 grams of cow dung slurry is inserted into the digester nine times to meet 4500 grams and another 170 grams to meet the 4670 grams. This action was done due to limited container is available in the lab.



**Figure 3.26:** 500 grams of cow dung slurry that marked by an orange strip.

After that, 4650 grams of cow dung slurry was inserted into three digesters accordingly as shown in Figure 3.27. The volume of cow dung slurry as inoculum in this study is fixed.



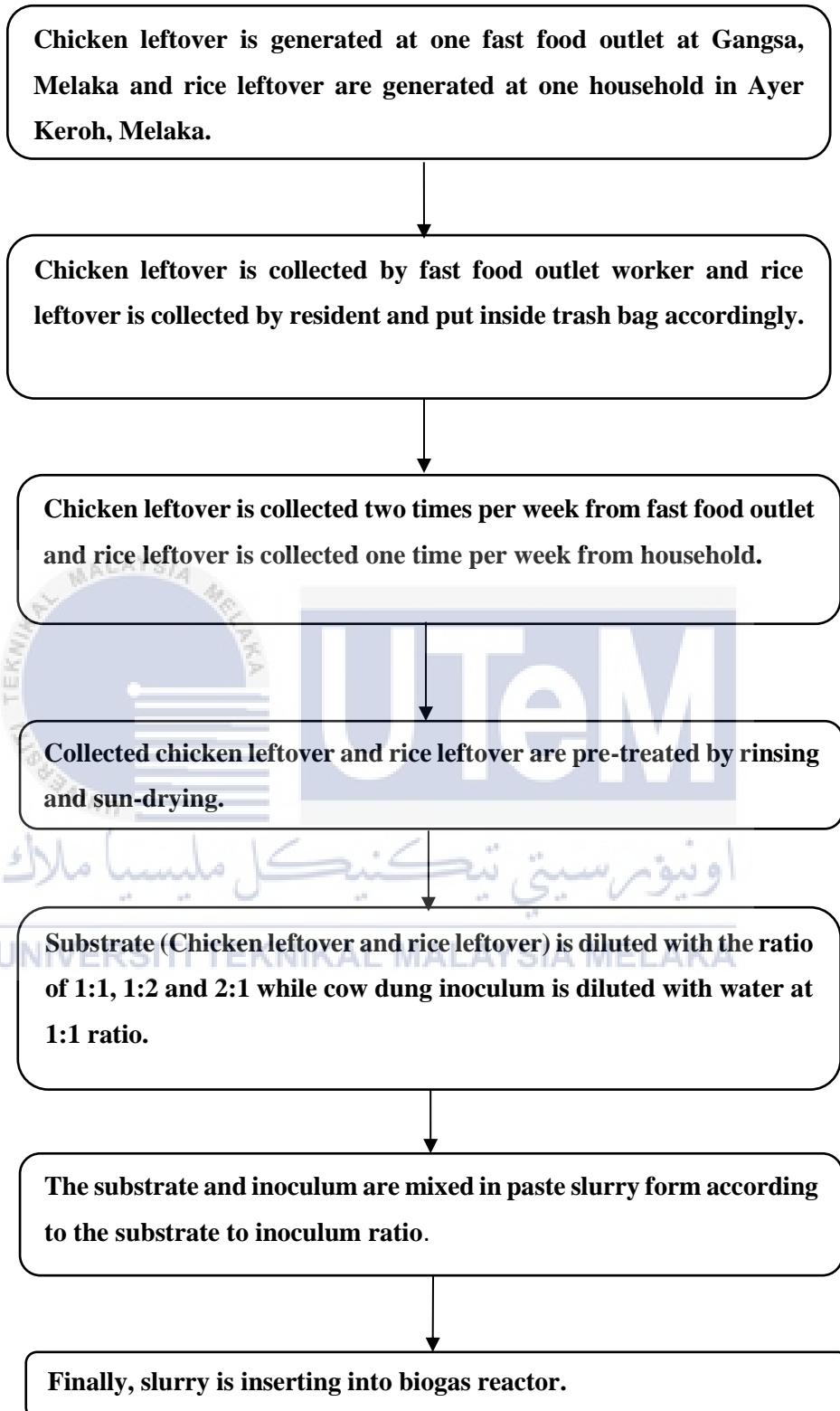
**Figure 3.27:** Cow dung slurry that was prepared to be inserted inside digester.

Lastly, substrate (chicken leftover and rice leftover) and inoculum (cow dung) were being inserted into three digesters with the chicken to rice ratio of 2:1, 1:1 and 1:2, respectively as shown in Figure 3.28.



**Figure 3.28:** Three digesters with the chicken to rice ratio of 2:1, 1:1 and 1:2 was placed in the Polymer Lab in FKP old building.

In this study, three anaerobic plastic bio-digesters with 25 litres volumes were constructed. Each of bio-digesters has fed separately with the chicken and rice ratio of 2:1, 1:1 and 1:2 and 4670 grams of cow dung as inoculums. Each ratio represents 184.5 grams. Fermentation slurry was prepared by adding and vigorous mixing of dried chicken leftover waste and dried rice leftover waste, with an equivalent amount of water needed for yield in the ratio of 2:1, 1:1 and 1:2. After feed in the slurry, the inlet of the bio-digester was immediately blocked with a pipe plug. Initial temperature was taken and found to be 37°C. Fermentation was allowed for a total period of twenty days which is ten days for cow fermentation and ten days fermentation for anaerobic digestion. The biogas reactor was stored in confined space in polymer lab which located at old FKP building to prevent light exposure. Figure 3.29 shows the step-by-step procedure for overall feedstock preparation in this study.



**Figure 3.29:** Overall feedstock preparation in this study.

### 3.2.2 Food Waste Analysis

This section is mainly discussed about the method to analyse commercial food waste (chicken leftover and rice leftover) using a Fourier Transform Infrared Spectroscopy (FTIR) analysis.

#### 3.2.2.1 Fourier Transform Infrared Spectroscopy (FTIR) analysis

Fourier Transform Infrared Spectroscopy (FTIR) was performed to identify the functional group of chicken waste leftover, rice waste leftover and cow dung and their correlated chemical composition. JASCO's FTIR machine was performed for spectroscopy analysis as shown in Figure 3.30 to confirm the presence of functional group and structural variations between chicken waste leftover and rice waste leftover. This FT-IR spectroscopy analysis was conducted at temperature of 25°C and was acquired at a resolution of 4.0 cm<sup>-1</sup> at a wave number range between of 400 cm<sup>-1</sup> to 4000 cm<sup>-1</sup> at a scanning speed of 2 mm<sup>-1</sup> at an aperture size of 7.1 mm. This analysis was carried out in accordance with the technique of attenuated total reflection (ATR).



**Figure 3.30:** FT-IR spectrophotometer (JASCO)

Figure 3.31 show the sample of dried chicken waste leftover and dried rice waste leftover for FTIR analysis. Then, the Attenuated Total Reflectance (ATR) sample setting in FTIR spectrometer as shown in Figure 3.32.

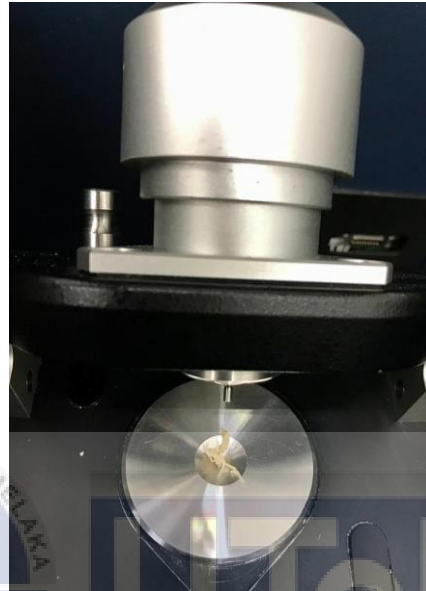


**Figure 3.31:** Dried chicken waste leftover and dried rice waste leftover for the FTIR analysis



**Figure 3.32:** ATR sample setting in FTIR spectrometer.

Figure 3.33 show the sample that undergoes the FTIR analysis. After ten minutes, the FTIR analysis result for the following sample is analyzed as shown in Figure 3.34.



**Figure 3.33:** Sample that undergoes FTIR analysis.



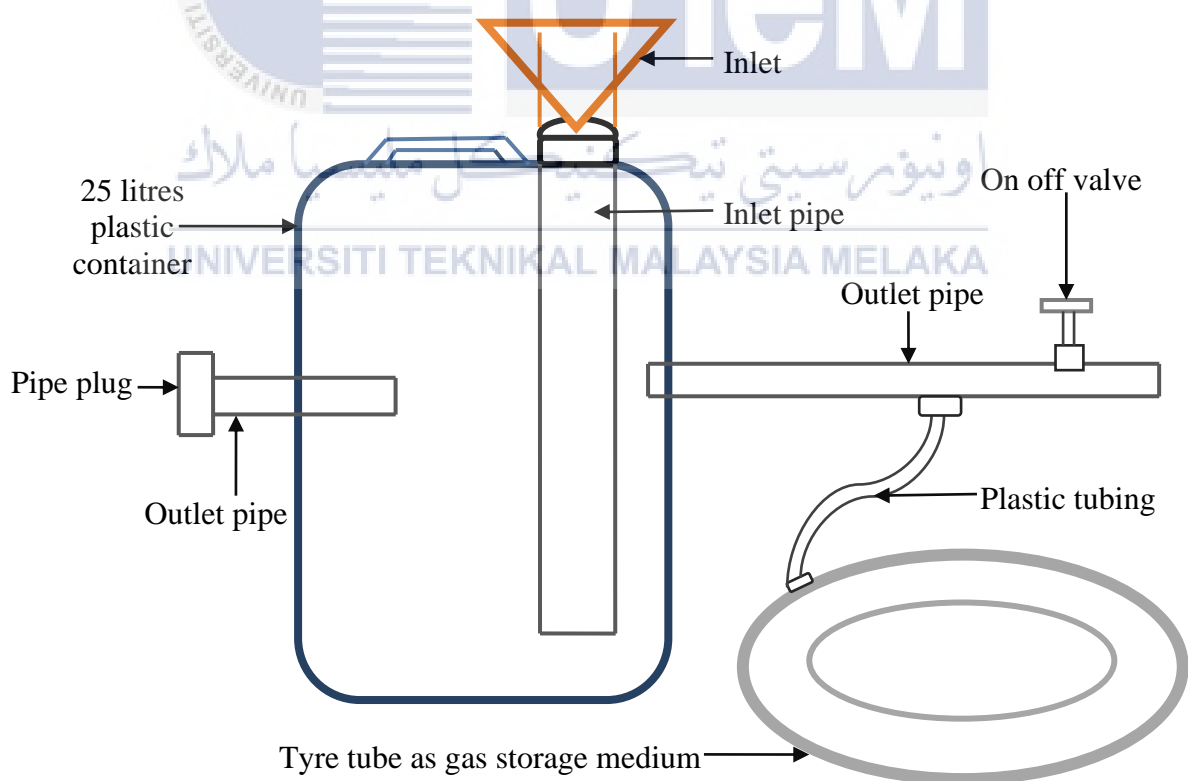
**Figure 3.34:** FTIR analysis result for the following sample is analyzed.

### 3.3 Biogas reactor Design

In this section, the schematic diagram of biogas mini-reactor design, materials and components required for produce the biogas reactor and the production process of biogas reactor were discussed.

#### 3.3.1 Schematic diagram

Figure 3.35 shows the schematic diagram for biogas mini-reactor tank designed in this study. The size of biogas reactor is 25-litres and the plastic container is attached to the input and outlet pipes, which are responsible for feeding in the slurry and removing the digested slurry. Outlet pipe is attached to the plastic container to allow the digested slurry to exit the on-off valve. Also, tyre tube as gas storage medium.



**Figure 3.35:** Schematic diagram for biogas reactor tank

### 3.3.2 Materials and components required

The materials that are used to construct the biogas mini-reactor are specified. Plastic container at a length of 42 cm with the volume capacity of 25 litres was used as main body part of biogas reactor, 80 mm PVC (Polyvinyl chloride) pipe as inlet pipe, 210 mm PVC (Polyvinyl chloride) pipe as outlet pipe, 280 mm, 380 mm and 400 mm plastic tubing as connection for gas collection system, 300 mm plastic tubing as gas delivery system, funnel as import tool for slurry, T-connector to connect plastic tubing (380 mm, 280 mm, 400 mm) and plastic tubing 300 mm and medium size tyre tube as gas storage medium. Superglue, fine sand, soldering iron, blade, and knife were utilized during the fabrication the biogas reactor. As well as, commercial food waste (chicken leftover and rice leftover), cow dung inoculum and tap water as mixture of feedstock.

### 3.3.3 Production Process

Experimental setup included a plastic container as biogas mini-reactor with a capacity of 25 litres. For feeding the industrial food waste and inoculums, PVC pipes was utilized, a guide pipe fixed with the biogas mini-reactor chamber and a pipe was used as the digested slurry outlet. Then, a smaller hole for the gas distribution system, a hole for feeding the slurry at inlet pipe and a hole for replacing the slurry at outlet pipe are made up of three holes. These holes are made with the aid of iron and blade soldering. In the upper half of the bottle, two holes are cut through the diametrically left side of the cylindrical body (one hole as connector for plastic tubing and another hole for inlet tube). In the middle of the bottle, one hole is cut through the right side of the cylindrical body as outlet pipe. Then, with the aid of fine sand and super glue, PVC pipes are attached to the hole.

Slurry consisting of water, industrial food waste (chicken leftover and rice leftover) and cow dung inoculum are loaded in the biogas reactor. To achieve anaerobic conditions, care was carefully taken to ensure zero air entry into the digester. Both the inlet and outlets are closed with pipe plug after the slurry filling. There are total of ten days for cow dung fermentation and ten days for anaerobic digestion. The expansion of tyre tube occurred



within the first three days of anaerobic digestion cycle due to output gas. The contents of the tyre tube were obtained after the end of twenty-day anaerobic period.

### 3.4 Output Quantification

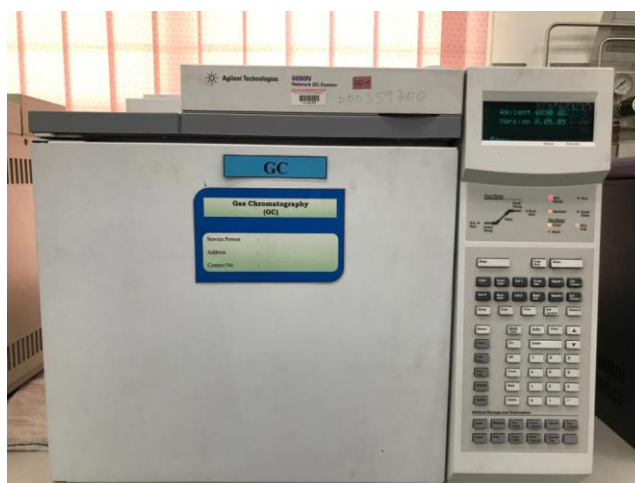
The output of commercial food waste source (chicken leftover waste and rice leftover waste), which is biohydrogen ( $H_2$ ) yield are quantified using Gas Chromatography-Thermal Conductivity Detector (GC-TCD). The analysis method is briefly explained at the following section.

#### 3.4.1 Biohydrogen ( $H_2$ ) Quantification

Biogas composition ( $CH_4$ ,  $CO_2$ ,  $H_2$  and  $N_2$  contents) was measured using a Gas Chromatography-Thermal Conductivity Detector (GC-TCD) (Model 6890-N) that available in Material Characterization laboratory in University Putra Malaysia (UPM) as shown in Figure 3.36 and Figure 3.37. Biogas samples were taken using a 100- $\mu$ l tight gas from the head space of the reactors after the gas was released from the tyre tube. The samples were inserted into the GC-TCD afterward.



**Figure 3.36:** GC-TCD gas analysis was performed at the material characterization lab of Universiti Putra Malaysia (UPM).



**Figure 3.37:** Gas Chromatography-Thermal Conductivity Detector (GC-TCD) (Model 6890-N)

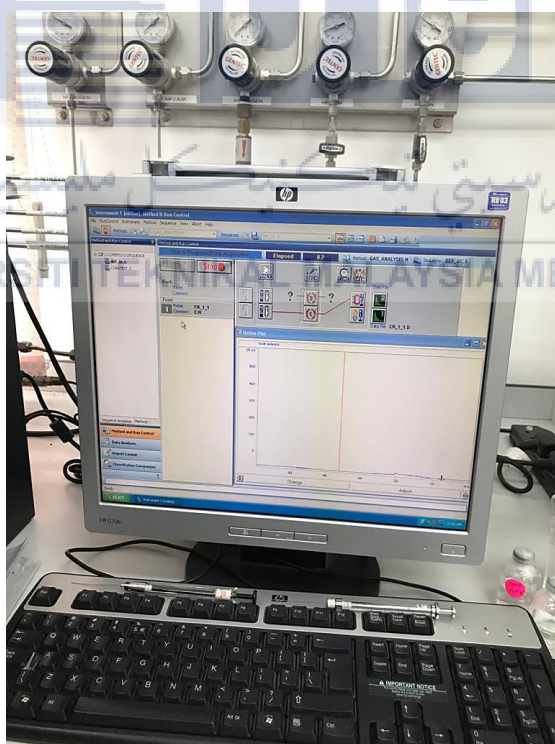
The syringe that filled with one millilitre of the sample is being injected into a septum in the injection port of the GC-TCD instrument for analysis as shown in Figure 3.39. After that, the compound sample is then being recorded as shown in Figure 3.40.



**Figure 3.38:** The sample that filled with biogas is being injected by a syringe.



**Figure 3.39:** The syringe that filled with one millilitre of the sample is being injected into a septum



**Figure 3.40:** The compound of the sample is then being recorded.

## CHAPTER 4

### RESULTS AND DISCUSSIONS

This chapter present and discuss the experimental results and design outcomes after the samples testing. All of the hypothesis and statement given were supported by other previous similar research with further justification and careful observation.

#### 4.1 Overview

The primary purpose of this research is to evaluate the potential of hydrogen production based on biogas mini-reactor design. From this study, we found that our reactor has the potential to generate biohydrogen gases. This can be seen when the chicken to rice ratio (2:1) substrate is able to produce 3.17608% of biohydrogen and the chicken to rice ratio (1:2) is able to produce a higher percentage of biohydrogen, which is 10.84388%. However, the biohydrogen in the chicken to rice ratio (1:1) sample is not detected the formation of biohydrogen. Since the total input weight for the chicken to rice (1:1) sample is only 369 grams, the input weight for the ratio of one for chicken leftover waste and one for rice leftover waste is insufficient to produce hydrogen. To overcome this, we believe by improving the selection of substrate ratio between the rice waste leftover and chicken waste leftover, and also evaluating the potential of biohydrogen generated by varying inoculum content, the biohydrogen would yield in these three samples.

The Fourier Transform Infrared (FTIR) analysis was utilized to analyse the chemical composition of specific food waste (chicken leftover and rice leftover) and cow dung inoculum, which, in this case, carbohydrate, protein (amide I), protein (amide II), fat, amino-related components, and water have been detected. Based on the observations, the chicken to rice (2:1) sample possessed higher concentration of carbohydrate content as compared than the other two samples. This is because, by covering white flour on top of chicken skin layer, the carbohydrate content of chicken to rice ratio (2:1) was found to increase. Actually, another technique is being used to support the data in this study, and that is Bomb Calorimetry analysis, which is used to determine the energy content of the samples. The ignite wire for Bomb Calorimetry has been bought, and the process for accessing the Faculty Mechanical lab is time-consuming. As a result of the movement control order restriction, the Bomb Calorimetry testing was eventually forced to be removed.

On the other hand, the Gas Chromatography-Thermal Conductivity Detector (GC-TCD) was utilized to detect the presence of gases in the chicken to rice ratio (1:1) sample, chicken to rice (1:2) sample and chicken to rice ratio (2:1) sample. Finally, several inorganic gases were detected under the observation of GC-TCD. There are hydrogen, methane, oxygen, nitrogen and carbon dioxide gases were detected. Each gas was analysed in terms of their retention time and area of peak in order to identify the most detected gases in these three samples. Related to that, it was found that there was an absence of methane gases in these three samples. This was due to insufficient anaerobic microbial activity as the fermentation of cow dung inoculum only took ten days. According to G. Zeeman (2003), a retention time of at least 50 days is necessary for methane production. Hence, there is no methane production due to total of twenty days of anaerobic digestion process that includes ten days of cow dung fermentation. This phenomenon has occurred due to limitation of time as the execution of Movement Control Order (MCO) since March 2020. Other than that, the biogas reactor and components parts for biogas mini-reactor was designed using Autodesk Inventor Pro 2020. There are a total of twelve important parts components for the biogas reactor and it was found that the most important part for biogas reactor is the plastic container that acted as main body for biogas reactor itself. It has the function of placing the slurry, which, in this case, is chicken waste leftover, rice waste leftover, and cow dung inoculum.

Lastly, our design is found potentially able to produce biohydrogen gases. Also, this result is validated by GC-TCD gas analysis. Biohydrogen generation has value added benefit

of not harmful to environment. Biohydrogen is thought to be one of next-generation biofuels, which has the potential to reduce fossil fuel reliance while also lowering greenhouse gas emissions from the energy and transportation sectors.

## 4.2 Feedstock Characterization

### 4.2.1 Fourier-transform Infrared Spectroscopy (FTIR)

By applying infrared radiation (IR) to samples of materials at different wavelengths, FTIR analysis evaluates a sample's absorbance or transmittance of infrared light. This is done to determine the substance's molecular structure. Raw data from a broad-band light source is converted into absorbance values at each wavelength by the Fourier transform spectrometer (Yu Qin, 2018).

The wavelength of an infrared spectrum is shown on the x-axis, which represents the intensity of the spectrum. The peaks, also called as absorbance bands, correspond to the different vibrations of the sample's atoms when exposed to the infrared part of the electromagnetic spectrum. The quantity of infrared light absorbed or transmitted by the substance being examined, on the other hand, is shown on the y-axis.

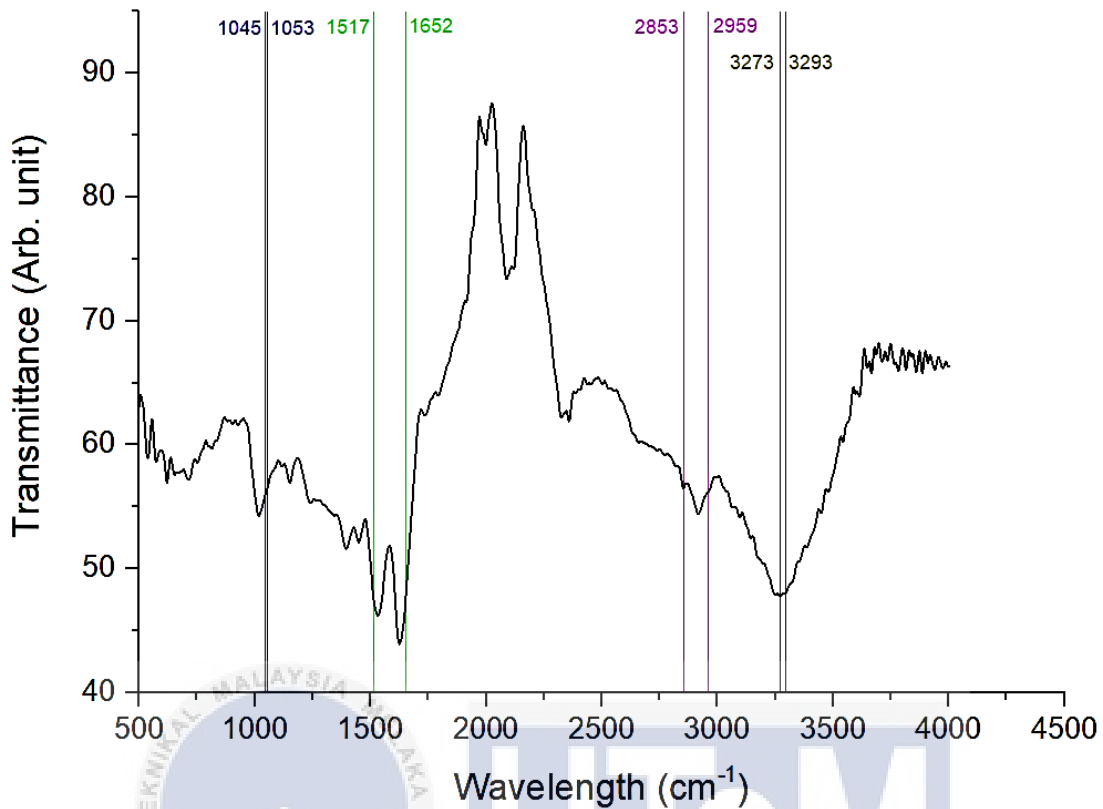
The data regarding the chemical components and physical state of the chicken waste leftover, which serves as one of the substrate component in this experiment, are shown in Figure 4.1 and Table 4.1. As seen in Figure 4.1, there are multiple colour lines with integer numbers on top. The colour line shows the chemical composition range of chicken waste leftover identified by FTIR spectrometer. The first gain peak had a wavelength range at between of  $1045\text{ cm}^{-1}$  to  $1053\text{ cm}^{-1}$  and was identified as a carbohydrate peak. This carbohydrate peak is the first peak from the left and is regarded as a sharp peak. This is consistent with Mordechai et al (2001). It was discovered that the carbohydrate structure involves C-O stretching combined with C-O bending of the C-OH at this location.

The second obvious peak has wavelengths ranging at between of  $1517\text{ cm}^{-1}$  to  $1652\text{ cm}^{-1}$ . This peak's chemical component has discovered to be a protein peak. This protein peak

has two kinds of band assignments, which are amide I and amide II. There are two important peaks at the region of  $1517\text{ cm}^{-1}$  to  $1652\text{ cm}^{-1}$ , as seen in Figure 4.1. These two peaks are known as amides I and amides II. It was discovered that the stretched C=N, C=C, and C=N guanine structures were presented in amide I. Schulz and Baranska (2007) and Paluszkiwicz and Kowiatek (2001) had investigated the existence of C-C stretch in the phenyl structure of amide II. As can be seen, even though amide I and amide II were derived from the same chemical component (protein), their underlying chemical structures might be different (Schulz and Baranska, 2007).

The chemical component of fat, according to Yang et al. (2005), had a wavelength at between of  $2853\text{ cm}^{-1}$  to  $2859\text{ cm}^{-1}$ . As can be seen in Figure 4.1, the fat peak is referred to the third peak from the left. This fat component's chemical structure was discovered to include  $\text{CH}_2$  for lipids and the asymmetric  $\text{CH}_2$  stretching mode of the methylene chains in membrane lipids (Yang et al, 2005).

Water can be found in this chicken waste leftover. Figure 4.1 shows the fourth peak from the left, with a wavelength range of  $3273\text{ cm}^{-1}$  to  $3293\text{ cm}^{-1}$ . According to Schulz and Baranska (2007), there is a stretched O-H symmetric structure within the water component.



**Figure 4.1:** Fourier-transform Infrared Spectroscopy (FTIR) analysis for Chicken Waste Leftover.

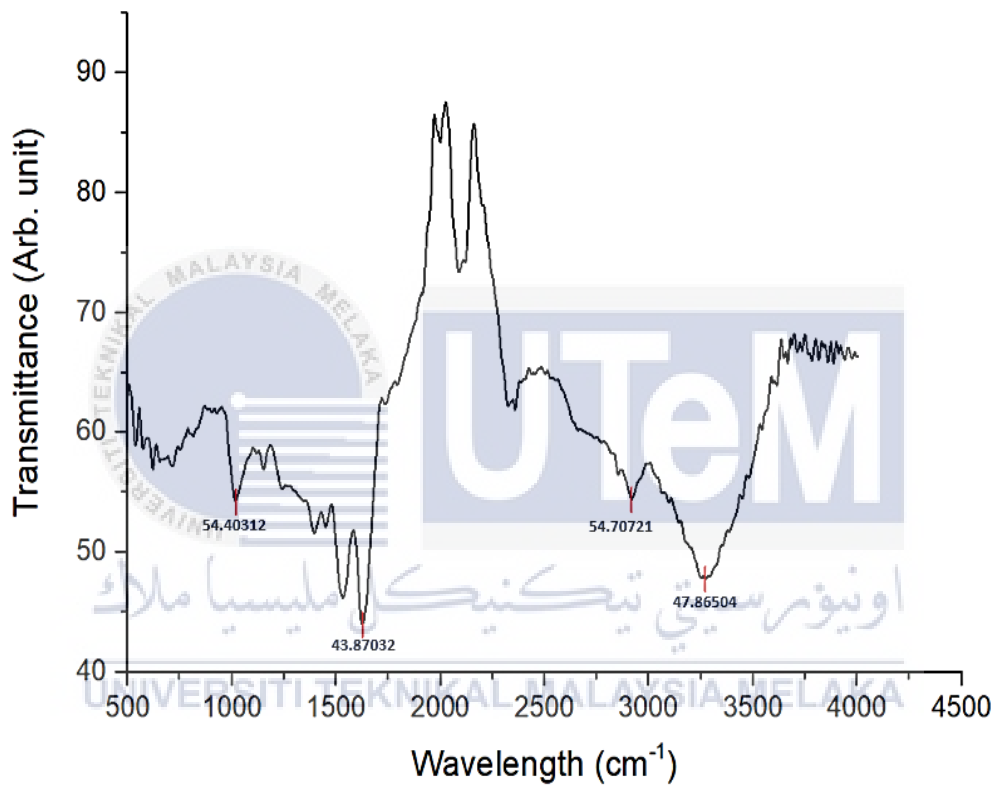
**Table 4.1:** Basic information regarding band assignment for the stated range in Figure 4.1.

| RANGE     | COMPONENT                                    | REFERENCES   |
|-----------|--|--|
| 1045-1053 | Carbohydrate                                 | Mordechai, 2001  |
| 1517-1652 | Protein (Amide I)<br>&<br>Protein (Amide II) | Schulz and Baranska, 2007<br>Paluszkiewicz and Kwiatek, 2001 |
| 2853-2959 | Fat  | Yang, 2005   |
| 3273-3293 | Water  | Schulz and Baranska, 2007                                    |

The peak location for chemical component of the chicken waste leftover sample described in Figure 4.1 is shown in Figure 4.2. As illustrated in Figure 4.2, there are four peaks from left to right: carbohydrates, protein (amide I), protein (amide II), fat, and water. In Figure 4.2, each of them has a peak location that is denoted by a red line. The peak position unit is labelled in percentages based on the transmittance on the y-axis.



It has a high ranking of 54.40312 for carbohydrates. Protein (amide I) and protein (amide II) acquired 43.87032 of their peak position for the following peak. Table 4.2 demonstrates this. Protein (amide I) and protein (amide II) constituents have the lowest peak position percentages, ranking fourth behind carbohydrates, protein (amide I), protein (amide I), and fat constituents. In comparison to the other components, the fat peak has the greatest peak position at 54.70721. And the water peak is at 47.86504 of its maximum position.



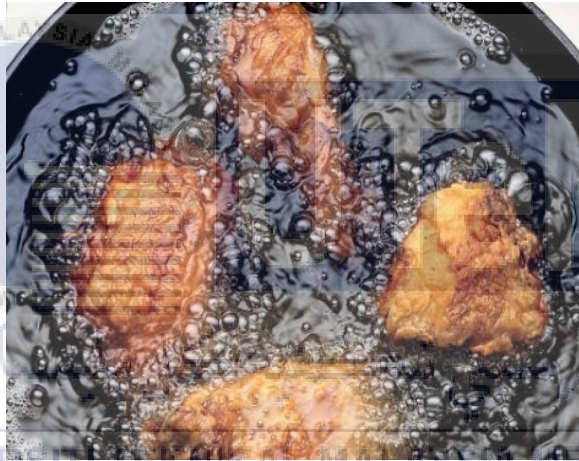
**Figure 4.2:** Peak position for carbohydrates, protein (amide I), protein (amide II), fat and water in the chicken waste leftover sample.

**Table 4.2:** Some information regarding the peak position and chemical component in the chicken waste leftover sample.

| PEAK POSITION | COMPONENT                              |
|---------------|--|
| 54.40312      | Carbohydrate                           |
| 43.87032      | Protein (Amide I) & Protein (Amide II) |
| 54.70721      | Fat                                    |
| 47.86504      | Water                                  |



**Figure 4.3:** Structure of Chicken waste leftover



**Figure 4.4:** Deep fried chicken (Sources: <https://stuffoholics.com/how-long-to-deep-fry-chicken-legs/>, access on: 20 August 2021).

The structure of the chicken residual waste is shown in Figure 4.3 above. Fourier-transform Infrared Spectroscopy (FTIR) has identified about four major chemical compositions: carbohydrates, protein (amide I) and protein (amide II), fat, and water. The abundance of fat and carbohydrates in chicken faeces is thought to originate from flour and oil. This is visible when the chicken is coated with flour and cooked in high-temperature oil, as illustrated in Figure 4.4. According to Myers AS (2012), the fat content of meals rises as a result of oil absorption. The presence of protein (amide I) and protein (amide II) as well as water is thought to have been produced by the chicken meat itself. Hassan Mohammad (2020)

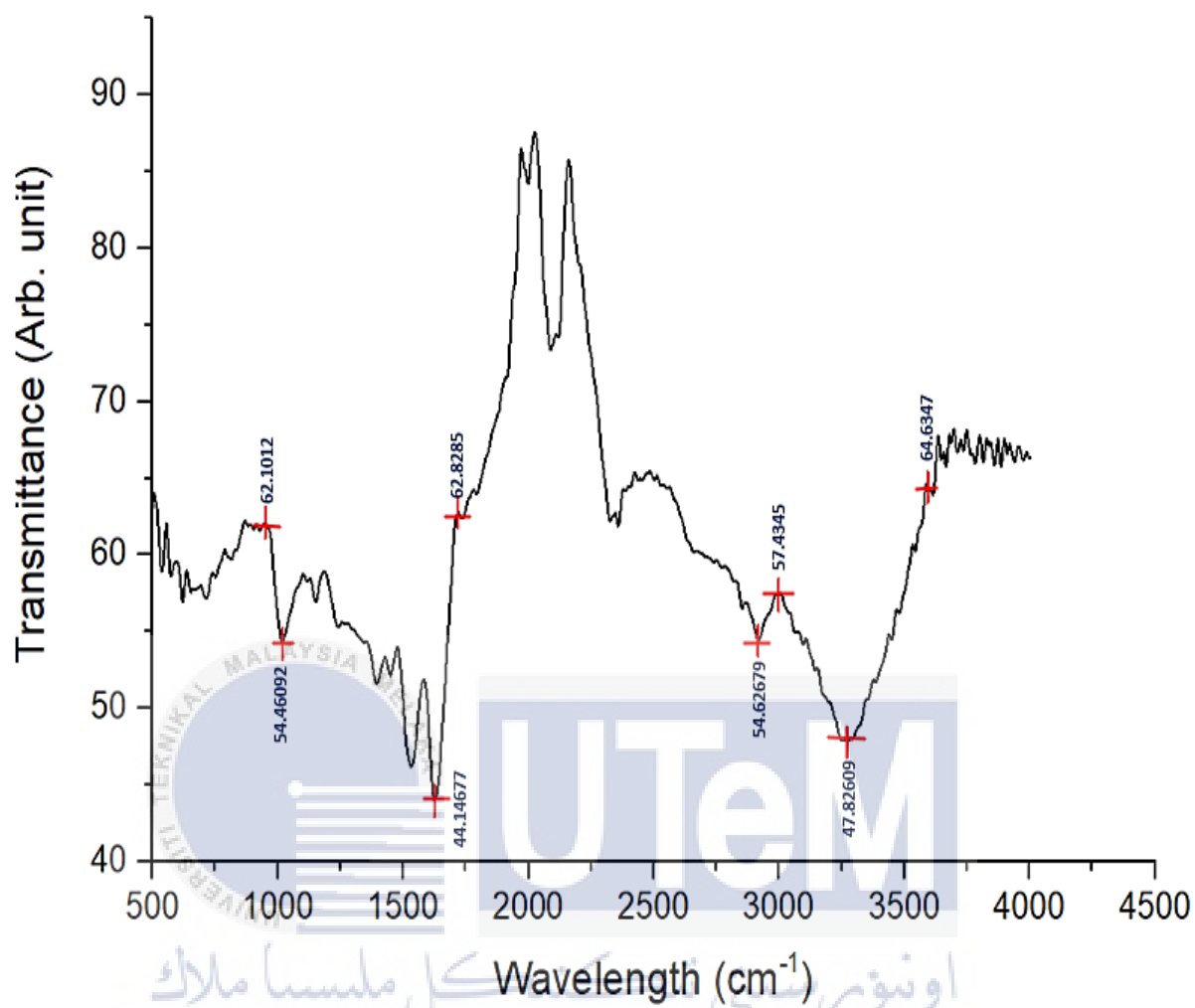
had explained this situation. It was discovered that the chicken breast itself had higher moisture content of 72.8 % and higher protein content 20.02 %.

The highest intensities of carbohydrate peak, protein (amide I) and protein (amide II) peaks, fat peak, and water peak are shown in Figure 4.5. Peak intensity is defined as the region concentrated peak. There are numerous red cross markings on various summits, as seen in Figure 4.5. The top and bottom values of the peak are shown by the red cross mark. The carbohydrates peak is displayed in Figure 4.5 as the first peak from the left. The highest figure for its peak position is 62.1012, while the lowest number is 54.46092. As a result, the overall peak intensity value is given by the difference between these two peak location values. The overall peak intensity value for this carbohydrate peak is 7.64028.

The overall peak intensity value for the protein (amide I) and protein (amide II) peaks, which are situated in the second position from the left following the carbohydrates peak, is 18.68173. This is because the highest peak position value of 62.8285 differs from the bottom peak position value of 44.14677. Protein (amide I) and protein (amide II) are placed first in Table 4.3 because their overall peak intensity value is the greatest when compared to the others.

The fat component with the lowest overall peak intensity value, on the other hand, is rated fourth. As a result, the overall concentration of fat content in the chicken leftover trash sample was determined to be low. Table 4.3 demonstrates this. The fat component's overall peak intensity value is 2.80771. This is because the top peak position value of 57.4345 differs from the bottom peak position value of 54.62679.

The peak position for the water component, on the other hand, is the fourth peak from the left. The highest peak position value for this water component is 64.6347, while the lowest peak position value is 47.82609, as given in Table 4.3. The difference between these two peak intensity values yielded a total peak intensity value of 16.80861, ranking second only to protein (amide I) and protein (amide II).



**Figure 4.5:** Peak intensity for carbohydrates, protein (amide I), amide (II) and fat component in the chicken waste leftover sample.

**Table 4.3:** Peak intensity information for carbohydrates, protein (amide I), amide (II) and fat component in the chicken waste leftover sample.

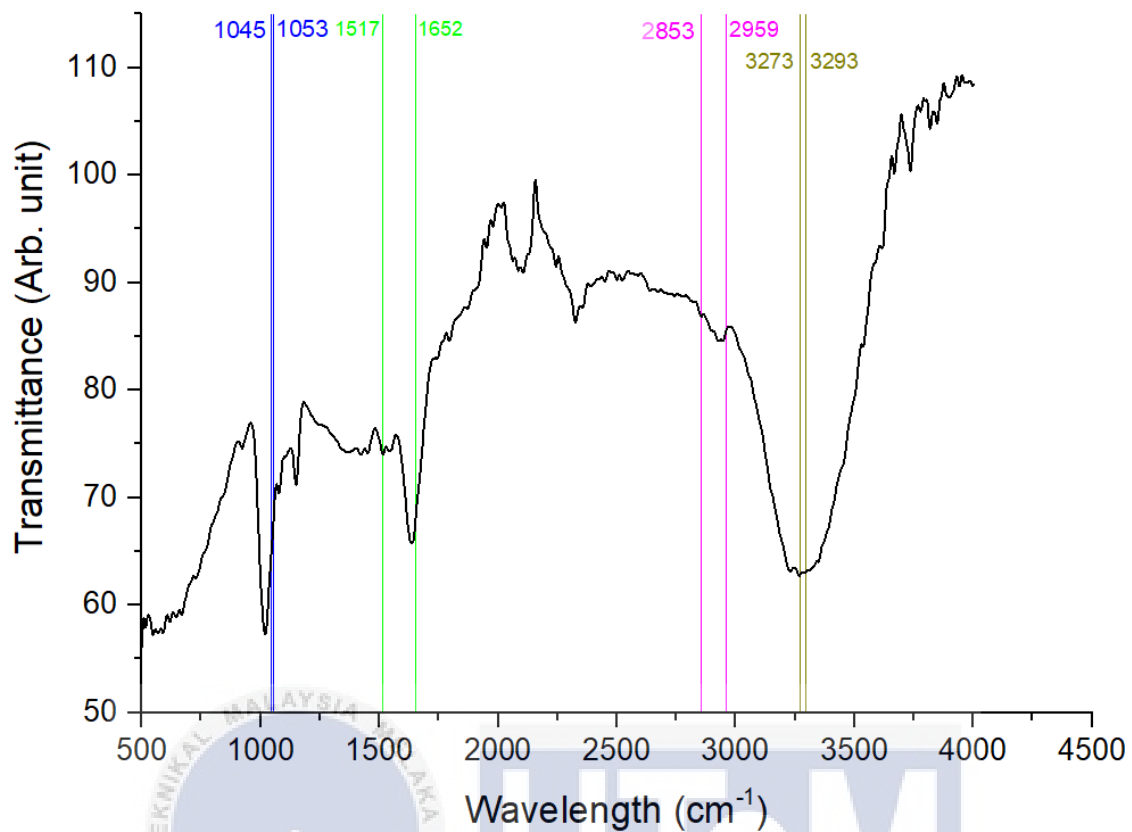
| PEAK INTENSITY   | TOTAL PEAK INTENSITY | COMPONENT                              |
|------------------|----------------------|--|
| 62.1012-54.46092 | 7.64028              | Carbohydrate                           |
| 62.8285-44.14677 | 18.68173             | Protein (Amide I) & Protein (Amide II) |
| 57.4345-54.62679 | 2.80771              | Fat                                    |
| 64.6347-47.82609 | 16.80861             | Water                                  |

The results regarding the chemical components and physical state of the rice leftover waste, which serves as one of the substrates in this experiment, are shown in Figure 4.6 and Table 4.4. This sample has many peaks, however the identification of associated chemical composition peaks are using various references. The first peak had a wavelength range at between of  $1045\text{ cm}^{-1}$  to  $1053\text{ cm}^{-1}$  and was identified as carbohydrate peak. This carbohydrate peak is the first peak identified from the left and is regarded as a sharp peak. This is consistent with Mordechai et al (2001). It was discovered that a C-O-C stretching structure including nucleic acid and phospholipids was present at this location Mordechai et al (2001).

The second peak has wavelengths ranging from  $1517\text{ cm}^{-1}$  to  $1652\text{ cm}^{-1}$ . This peak's chemical component was discovered to be a protein peak. This protein peak has two kinds of assignments, amide I and amide II. There are two peaks in the range of  $1517\text{ cm}^{-1}$  to  $1652\text{ cm}^{-1}$ , as seen in Figure 4.6. These two peaks are known as amides I and II. The presence of C=O, stretching C=C uracyl, and NH<sub>2</sub> guanine peptide was discovered in amide I. The amide II, on the other hand, has a C-C stretch in the phenyl structure. As can be seen, even though amide I and amide II are derived from the same chemical component (protein), their underlying chemical structures may be quite different. According to Schulz and Baranska (2007) and Paluszkiwicz and Kwiatek (2001).

The chemical component of fat, according to Yang et al. (2005), has a wavelength of  $2853\text{ cm}^{-1}$  to  $2859\text{ cm}^{-1}$ . Also, as seen in Figure 4.6, the fat peak is the third peak from the left. This fat component's chemical structure was discovered to include CH<sub>3</sub> of lipids, DNA, and proteins and the asymmetric stretching mode of cellular protein methyl groups.

Water can be found in this chicken leftover waste. Figure 4.6 shows the fourth peak from the left, with a wavelength range of  $3273\text{ cm}^{-1}$  to  $3293\text{ cm}^{-1}$ . According to Schulz and Baranska (2007), there is a stretched O-H symmetric structure within the water component.



**Figure 4.6:** Fourier-transform Infrared Spectroscopy (FTIR) analysis for Rice Waste Leftover.

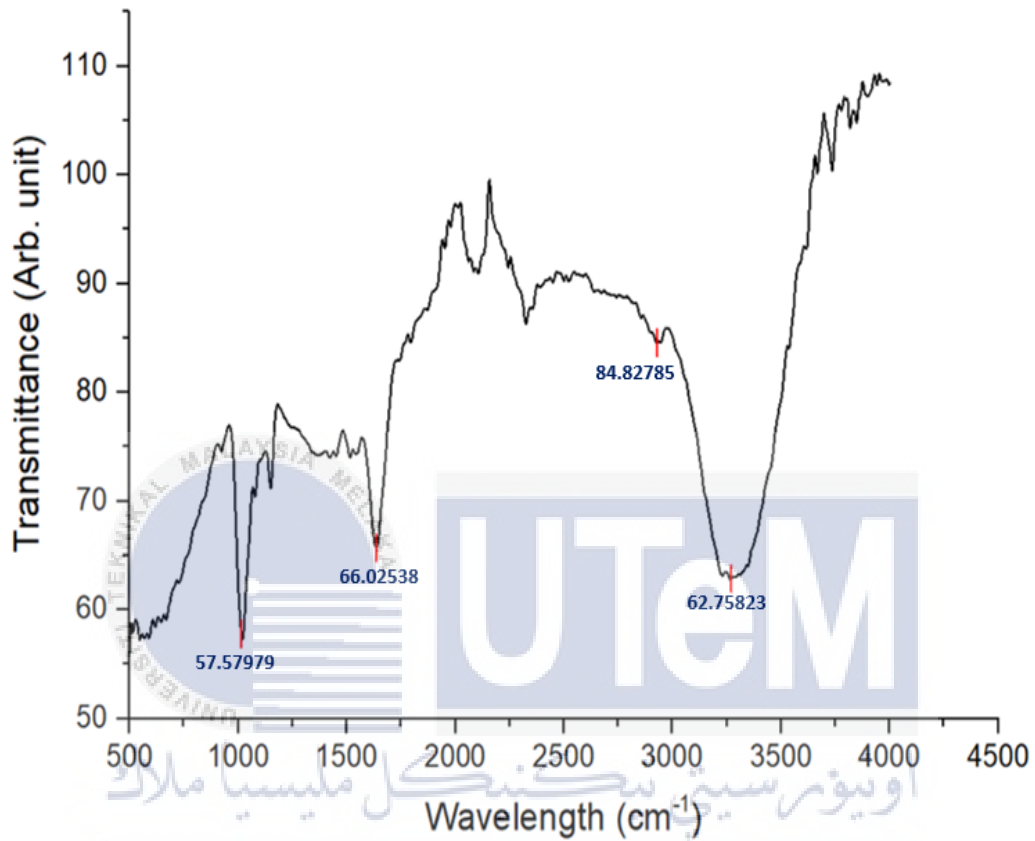
**Table 4.4:** Basic information regarding band assignment for the range that stated in the Figure 4.6.

| RANGE     | COMPONENT                                    | REFERENCES   |
|-----------|--|--|
| 1045-1053 | Carbohydrate                                 | Mordechai, 2001  |
| 1517-1652 | Protein (Amide I)<br>&<br>Protein (Amide II) | Schulz and Baranska, 2007<br><br>Paluszkiewicz and Kwiatek, 2001 |
| 2853-2959 | Fat  | Yang, 2005   |
| 3273-3293 | Water  | Schulz and Baranska, 2007  |

The peak location for the chemical component of the rice residual waste sample, as described in Figure 4.6, is shown in Figure 4.7. From left to right, there are four peaks labelled carbohydrates, protein (amide I), protein (amide II), fat, and water. In Figure 4.7, each of them has a peak location that is denoted by a red line. The peak position unit is labelled based on the transmittance on the y-axis.

It has 57.57979 of its peak position for carbohydrates. Schulz and Baranska (2007) stated that protein (amide I) and protein (amide II) acquired 66.02538 of their peak position

for the following peak. Table 4.5 demonstrates this. Yang (2005) reported that the fat component has the greatest peak position, which is 84.82785, and therefore ranks first. The water peak, on the other hand, has a peak position of 62.75823, ranking third behind the fat component, protein (amide I), and protein (amide II).



**Figure 4.7:** Peak position for carbohydrates, protein (amide I), protein (amide II), fat and water in the rice waste leftover sample.

**Table 4.5:** Some information regarding the peak position and chemical component in the rice waste leftover sample.

| PEAK POSITION | COMPONENT                                    | REFERENCES  |
|---------------|--|---|
| 57.57979      | Carbohydrate                                 | Mordechai, 2001   |
| 66.02538      | Protein (Amide I)<br>&<br>Protein (Amide II) | Schulz and Baranska, 2007<br>Paluszkiwicz and Kwiatek, 2001 |
| 84.82785      | Fat  | Yang, 2005  |
| 62.75823      | Water  | Schulz and Baranska, 2007                                   |

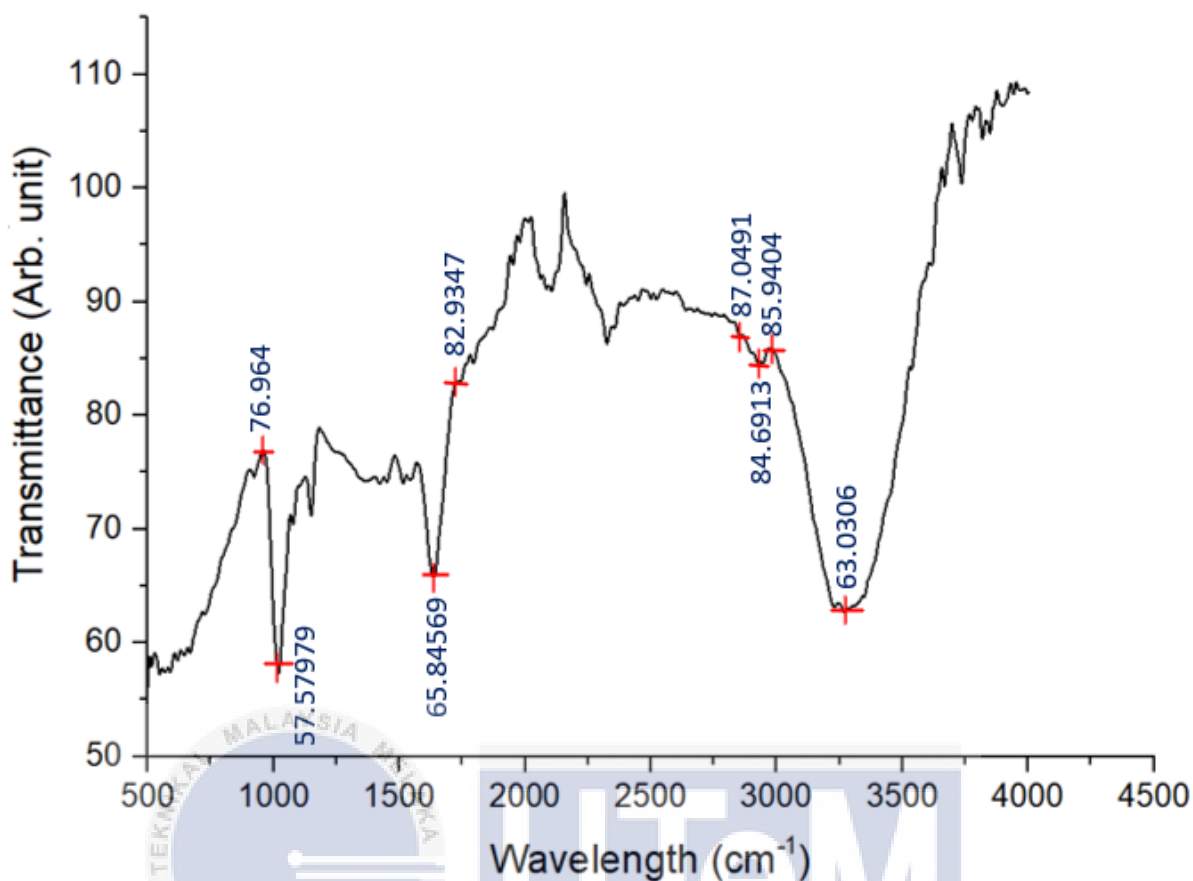
Figure 4.8 depicts the carbohydrate peak, protein (amide I) and protein (amide II) peaks, fat peak, and water peak intensity for rice leftover waste. According to Mordechai, (2001), peak intensity is defined as the region of concentration for a certain peak. There are numerous red cross markings on various summits, as seen in Figure 4.8. The top and bottom values of the peak are shown by the red cross mark. The carbohydrates peak is displayed in Figure 4.8 as the first peak from the left. The highest figure for its peak position is 76.964, while the lowest number is 57.57979. As a result, the overall peak intensity value is given by the difference between these two peak location values. The overall peak intensity value for this carbohydrate peak is 19.38421, and it was discovered that it ranks second after the water peak.

The overall peak intensity value for the protein (amide I) and protein (amide II) peaks, which are situated in the second position from the left following the carbohydrates peak, is 17.08901. This is because the top peak position value of 82.9347 differs from the bottom peak position value of 65.84569. As seen in Table 4.6, protein (amide I) and protein (amide II) are placed third, after the water and carbohydrate peaks.

The fat component with the lowest overall peak intensity value, on the other hand, is rated fourth. As a result, the overall concentration of fat content in the chicken leftover trash sample was determined to be low. Table 4.6 illustrates this. The fat component's overall peak intensity value is 2.3578. This is because the top peak position value of 87.0491 differs from the bottom peak position value of 84.6913.

Schulz and Baranska (2007) has reported that the peak position for water component, on the other hand, is the fourth peak from the left. The highest peak position value for this water component is 85.9404, while the lowest peak position value is 63.0306. Table 4.6 illustrates this. The disparities between these two peak intensity values have resulted in a total peak intensity value of 22.9098, which ranks first among the others since the total peak intensity value is the highest.





**Figure 4.8:** Peak intensity for carbohydrates, protein (amide I), amide (II) and fat component in the rice waste leftover sample.

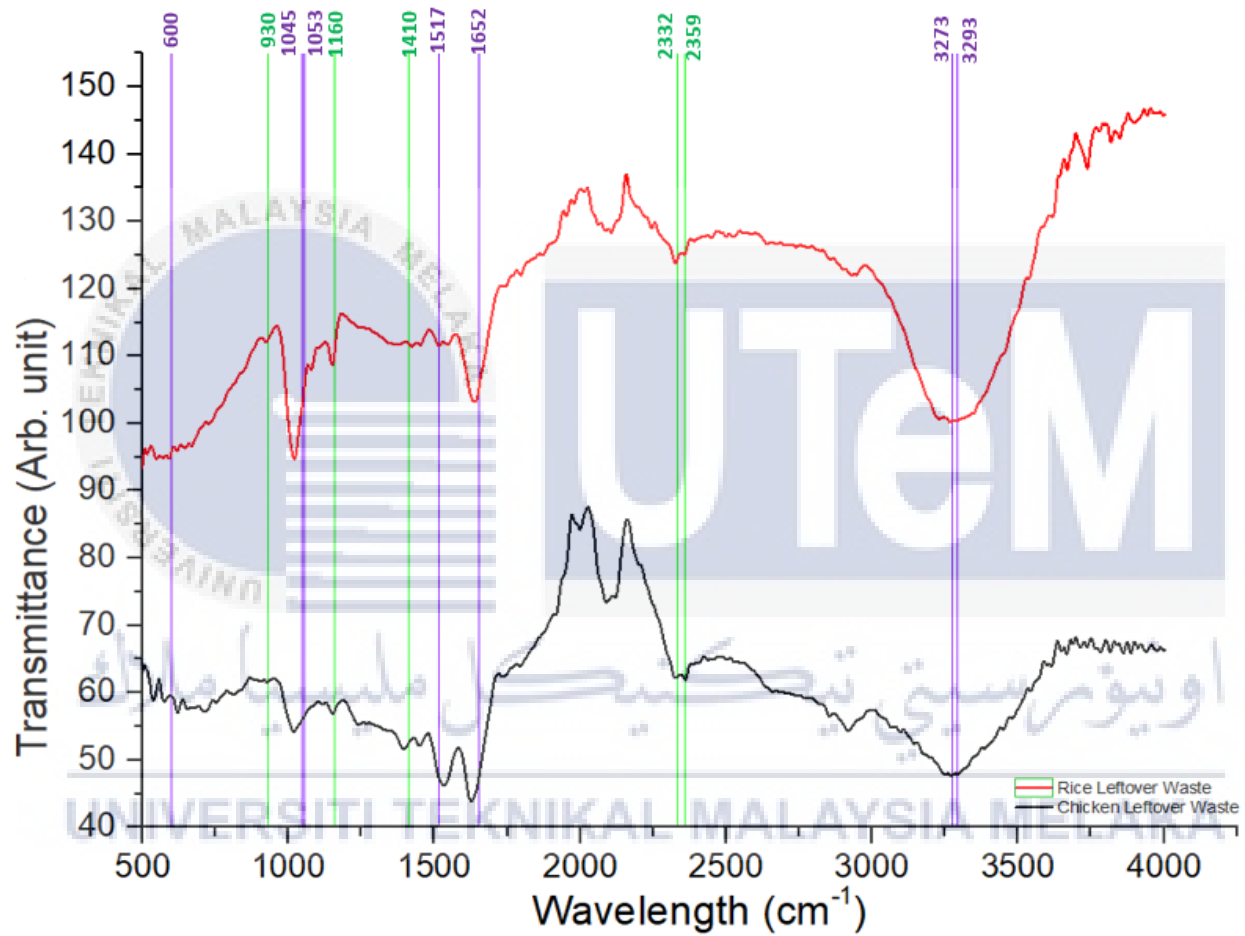
**Table 4.6:** Peak intensity information for carbohydrates, protein (amide I), amide (II) and fat component in the rice waste leftover sample.

| PEAK INTENSITY   | TOTAL PEAK INTENSITY | COMPONENT                              |
|------------------|----------------------|--|
| 76.964-57.57979  | 19.38421             | Carbohydrate                           |
| 82.9347-65.84569 | 17.08901             | Protein (Amide I) & Protein (Amide II) |
| 87.0491-84.6913  | 2.3578               | Fat                                    |
| 85.9404-63.0306  | 22.9098              | Water                                  |

The FTIR spectra in Figure 4.9 and functional group characteristics in Table 4.7 demonstrates the chemical differences between chicken waste leftover and rice waste

leftover. Chiang (1999) had stated that the CH out-of-plane bending vibrations from organic material of chicken waste leftover and rice waste leftover samples were given a wavelength of  $600\text{ cm}^{-1}$ . Nandiyanto et al. (2016) reported that the distinctive peaks at  $930\text{ cm}^{-1}$  were ascribed to carbon-induced ring deformation of the phenyl carbon-related component. According to Huleihel. (2002), the carbohydrates component was given the C-O stretching combined with C-O bending of the C-OH of carbohydrates at  $1045\text{-}1053\text{ cm}^{-1}$ . The greater relative intensities of these two rice waste leftover peaks suggested that they contained more carbohydrate than chicken waste leftover.

On the other hand, Fujioka (2004) had mentioned that the absorption peaks at  $1160\text{ cm}^{-1}$  was generated from proteins (serine, threonine, and tyrosine) and collagen, mostly from the C-O stretching mode of C-OH groups of serine, threonine, and tyrosine. The relative intensities of these peaks were greater in the rice waste leftover sample, indicating that it had a larger protein content. In both samples, Nandiyanto (2016) reported that there is a carbon-related component from carbon at a wavelength of  $1410\text{ cm}^{-1}$ . As seen in Figure 4.9, the chicken waste leftover sample has a greater peak than the rice waste leftover sample. Paluszkiwicz and Kwiatek (2001) stated that stretching C=N, C=C, C=N guanine, and C-C stretch of phenyl resulted in absorption peaks at  $1517\text{-}1652\text{ cm}^{-1}$ , which were linked with protein amide I and amide II. Nandiyanto (2018b) mentioned that the NH component produced by the amino-related component was ascribed to the distinctive peaks at  $2332\text{-}2359\text{ cm}^{-1}$ . According to Doybeshko (2000), the absorption bands at  $2853\text{-}2959\text{ cm}^{-1}$  for the fat components in the samples were attributed to  $\text{CH}_2$  of lipids and the asymmetric  $\text{CH}_2$  stretching mode of the methylene chains in membrane lipids. Doybeshko (2000) also stated that the high-water content of rice leftover waste led to a massive distinctive peak in the  $3273\text{-}3293\text{ cm}^{-1}$  region.



**Figure 4.9:** Comparison between chicken waste leftover and rice waste leftover.

**Table 4.7:** Basic information regarding to the chicken waste leftover and rice waste leftover.

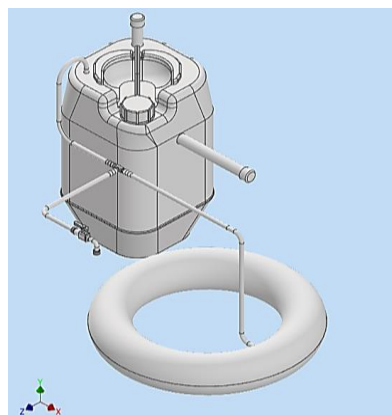
| Wavelength (cm <sup>-1</sup> ) | Functional group   | Band Assignment  | References                      |
|--------------------------------|--|--|---------------------------------|
| 600                            | CH out-of-plane bending vibrations   | Organic material                                       | Chiang., 1999                   |
| 930                            | Ring deformation of phenyl carbon-related component  | Carbon   | Nandiyanto, 2016                |
| 1045-1053                      | C-O stretching coupled with C-O bending of the C-OH of carbohydrates   | Carbohydrate   | Huleihel., 2002                 |
| 1160                           | Mainly from the C-O stretching mode of C-OH groups of serine, threonine, and tyrosine of proteins                | Protein (serine, threonine, and tyrosine) and collagen | Fujioka, 2004                   |
| 1410                           | Carbon-related component   | Carbon   | Nandiyanto, 2016,               |
| 1517-1652                      | Stretching C=N, C=C, C=N<br>guanine<br>C-C stretch of phenyl   | Protein (Amide II)<br>Protein (Amide I)                | Paluszkiewicz and Kwiatek, 2001 |
| 2332-2359                      | NH component   | Amino-related component                                | Nandiyanto, 2018b               |
| 2853-2959                      | CH <sub>2</sub> of lipids, Asymmetric CH <sub>2</sub> stretching mode of the methylene chains in membrane lipids | Fat  | Dovbeshko., 2000                |
| 3273-3293                      | Stretching O-H symmetric   | Water  | Dovbeshko., 2000                |

### 4.3 Reactor design

This section has gone through the detailed drawing of biogas mini-reactor, the assembly view of biogas mini-reactor, the exploded view of the biogas mini-reactor, and an explanation of the biogas mini-reactor's parts and components. The components of biogas mini-reactor in orthographic view, the materials and components needed in the production of biogas mini-reactor, and the biogas mini-reactor fabrication process were briefly described in this part.

#### 4.3.1 Detail Drawing

The detailed design of biogas mini-reactor is shown in Figure 4.10. The reactor design is referred based on the reactor design by Jyothilakshmi R (2016). In this study, the size of biogas mini-reactor is 25-litre with dimensions of 42 cm x 27 cm and dark blue translucent colour to allow for better anaerobic digestion and to prevent light from penetrating into it. The plastic container is then attached to input and outlet pipes, which are responsible for feeding in the slurry and removing the digested slurry. As a gas collecting system, the plastic container is also linked to the plastic tubing of 380 mm sizes, T connector, plastic connector 280 mm, plastic connector 400 mm, and tyre tube. Plastic tubing of 300 mm is attached to the plastic container to allow the digested slurry to exit the on-off valve. Autodesk Inventor Pro 2020 is used to design all of the components for the biogas reactor.



**Figure 4.10:** Detail drawing for the biogas mini-reactor

### 4.3.2 Views of the product

Figure 4.11 shows the assembly view of biogas mini-reactor in orthographic view which drawn with Autodesk Inventor Pro 2020. Top view, front view and side view of biogas mini-reactor has illustrated at the figure below.

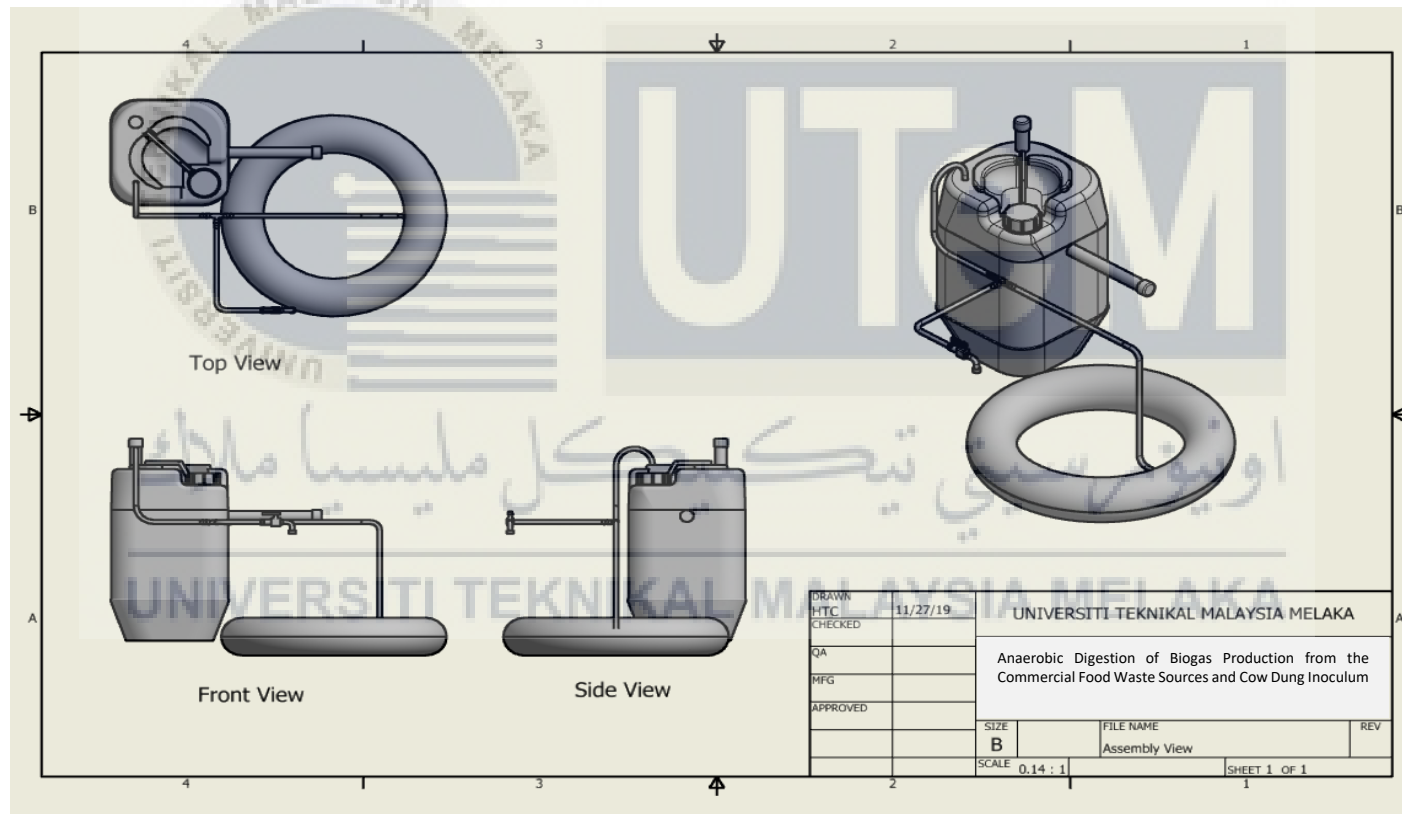


Figure 4.11: Assembly view of biogas mini-reactor in orthographic view

### 4.3.3 Exploded view

The biogas mini-reactor has total of twelve parts of main components which is one unit of plastic container, one unit of plastic container cap, one unit of inlet pipe, two unit of pipe plugs, one unit of outlet pipe, one unit of plastic tubing in the dimension of 380 mm, one unit of T connector, one unit of plastic tubing in the dimension of 280 mm, one unit of plastic tubing in the dimension of 300 mm, one unit of on off valve, one unit of plastic tubing in the dimension of 400 mm and one unit of tyre tube. Figure 4.12 shows the exploded view of the biogas reactor with the name in a proper form.

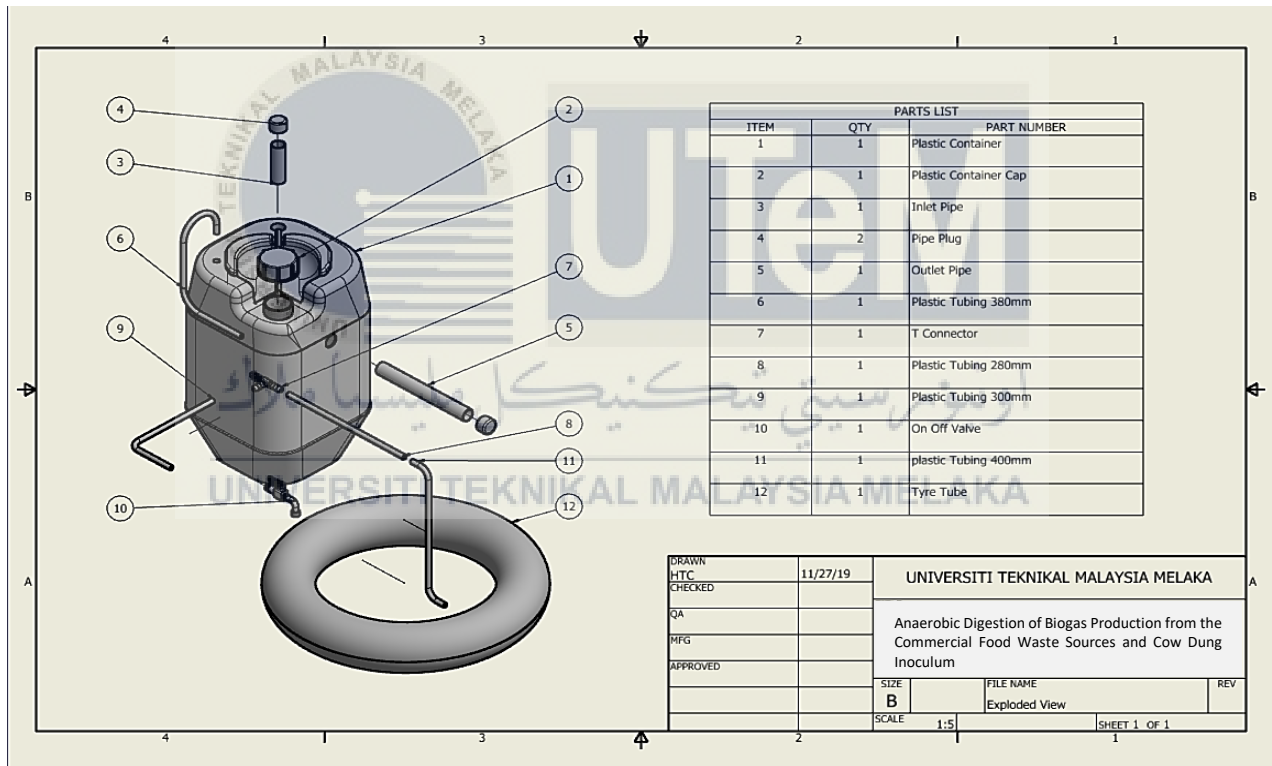


Figure 4.12: Exploded view of biogas mini-reactor in orthographic view.

#### 4.3.4 Product Components Functionalities

The biogas mini-reactor consists of many parts that are assembled together as completed product. Table 4.8 shows the components of biogas reactor and the function for each component. As referring to Table 4.8, there are total of 12 components that assemble together in order to make up a complete biogas mini-reactor. Nearly every component has the unit of one, but there are two pipe plugs is used in this case to seal up the inlet pipe and outlet pipe. For the part component number one, it named as plastic container that are dark blue in colour and is considered as the most important part in the making of biogas reactor. Plastic container can be said as the main body for biogas reactor itself. It has the function of place the slurry, which in this case, chicken leftover waste, rice leftover waste and cow dung inoculum. And then, there is an anaerobic digestion process undergoes inside this plastic container in the absence of oxygen and light.

Next, for the part component number two, it is the plastic container cap that function as to sealed up the plastic container which prevent the air flow into the plastic container. Inlet pipe and outlet pipe as part component number three and part component number five is connected to the plastic container and each have a function of feed in and flow out the digested slurry. For the part component number four, it is pipe plug and have the total unit of two. Pipe plug is function as to sealed up the inlet pipe and outlet pipe which ensure the airtight condition of biogas reactor. Then, there are also components that used to transfer the gases to the on off valve and the tyre tube. The components that have such function are the plastic tubing 280 mm, plastic tubing 300 mm, plastic tubing 380 mm and plastic tubing 400 mm. For instance, plastic tubing 280 mm, plastic tubing 380 mm and plastic tubing 400 mm is function as gas transfer system from the plastic container to the tyre tube.

On the other hand, the presence of T connector as part component number seven is used to connect all the plastic tubing that in 280 mm, 300 mm, 380 mm and 400 mm. For the part component number ten, that is the on off valve, have the function of flow out all the leftover digest slurry from the bottom of the plastic container. As for the tyre tube as part component number twelve, it has the function as collect all the gases that generated from the plastic container. And then, the collected gas that placed in the tyre tube will bring to Universiti Putra



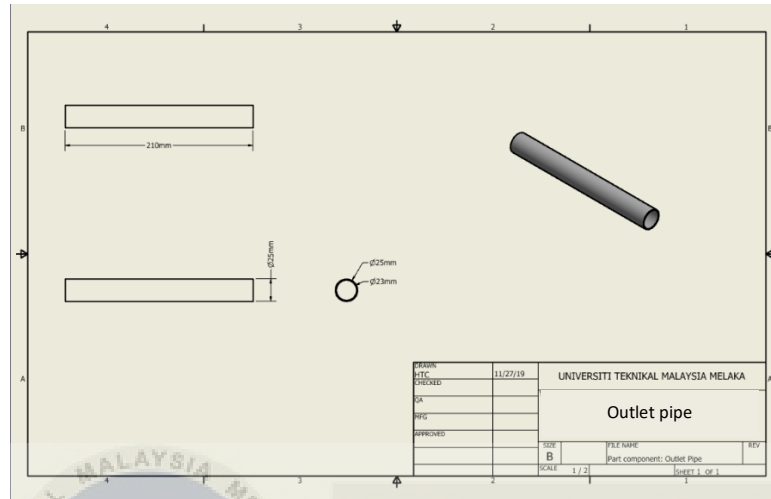
Malaysia (UPM) for Gas Chromatography -Thermal Conductivity Detector (GC-TCD) gas analysis.

**Table 4.8:** Part name of biogas reactor

| NO. | QTY. | PART NO. | PART NAME.            | FUNCTION   |
|-----|------|----------|-----------------------|--|
| 1   | 1    | 1        | Plastic Container     | As a biogas plant in which slurry is undergoes anaerobic digestion inside with absence of oxygen |
| 2   | 1    | 2        | Plastic Container Cap | Have to sealed to make sure airtight of plastic container  |
| 3   | 1    | 3        | Inlet Pipe            | To feed in the biological waste slurry   |
| 4   | 2    | 4        | Pipe Plug             | To off the inlet pipe and outlet pipe  |
| 5   | 1    | 5        | Outlet Pipe           | To let the digested slurry comes out from it.  |
| 6   | 1    | 6        | Plastic Tubing 380 mm | As a gas collection system   |
|     |      | 8        | Plastic Tubing 280 mm |  |
|     |      | 11       | Plastic Tubing 400 mm |  |
| 7   | 1    | 7        | T connector           | To connect plastic tubing (380 mm, 280 mm,400 mm) and plastic tubing 300 mm.                     |
| 8   | 1    | 9        | Plastic Tubing 300 mm | As a gas delivery system   |
| 9   | 1    | 10       | On Off Valve          | To let the leftover digested slurry comes out from it.   |
| 10  | 1    | 12       | Tyre Tube             | As a gas storage tank  |

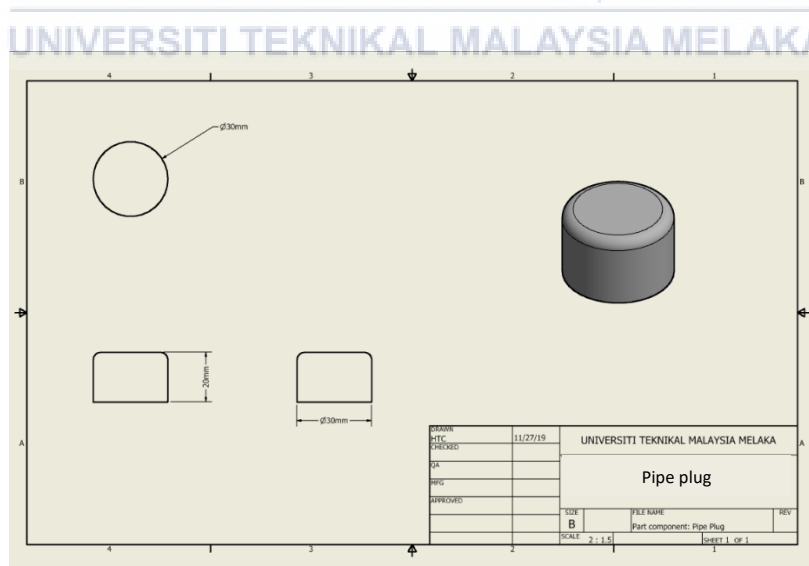


Based on Figure 4.15, the dimension of an outlet pipe is 210 mm and the diameter is 25 mm. The outlet pipe is made up of Polyvinyl Chloride (PVC) material and is connected to the plastic container and pipe plug. It functions as let the digested slurry comes out from it.



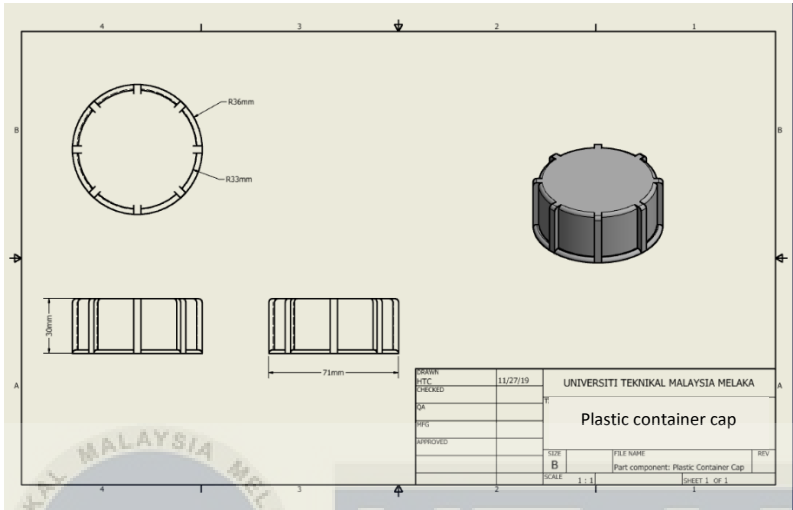
**Figure 4.15:** Outlet pipe

Figure 4.16 below shows the design of pipe plug of the product. The pipe plug is made up of Polyvinyl Chloride (PVC) material and is connected to the inlet pipe and outlet pipe. It functions as to seal the inlet pipe and outlet pipe. The pipe plug has a diameter of 30 mm, the height is 20 mm and the width is 30 mm.



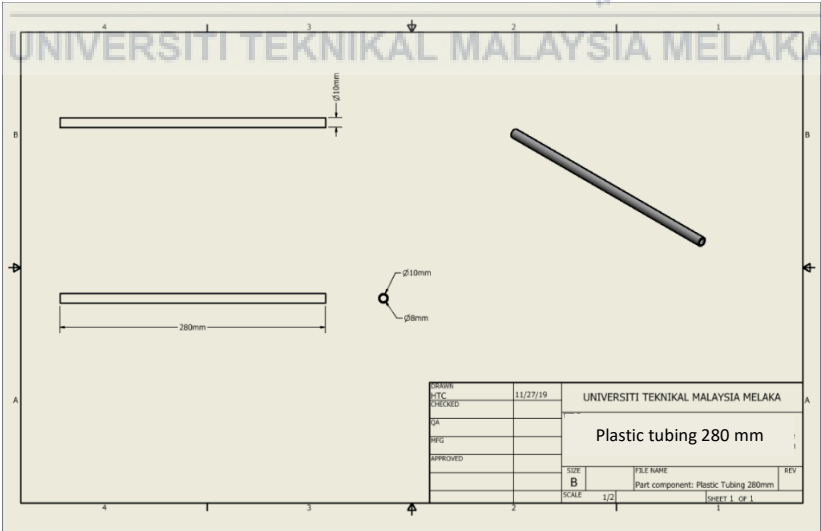
**Figure 4.16:** Pipe plug

Based on Figure 4.17 which is the plastic container cap, the dimension for the tank is 30 mm in length and 71 mm wide. The plastic container cap is 36 mm in diameter. It functions as to sealed the plastic container.



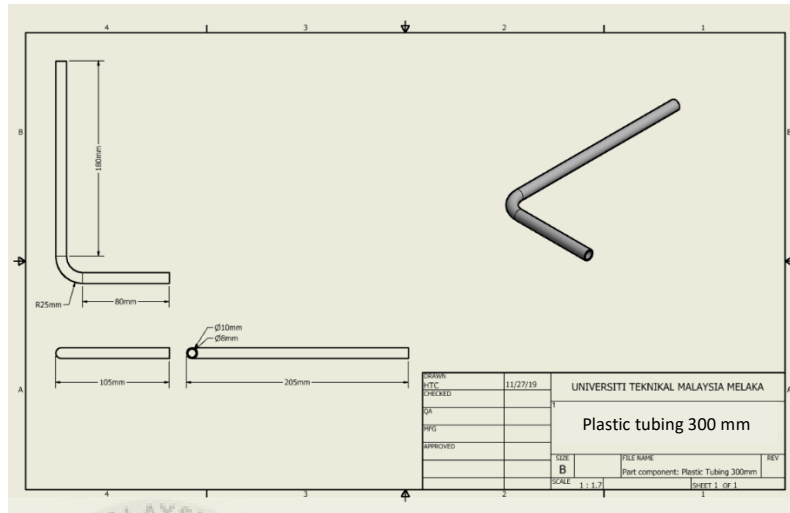
**Figure 4.17:** Plastic container cap

Based on Figure 4.18, the dimension for the plastic tubing is 280 mm and the diameter is 10 mm. The plastic tubing is connected to the T connector and plastic tubing 400 mm.



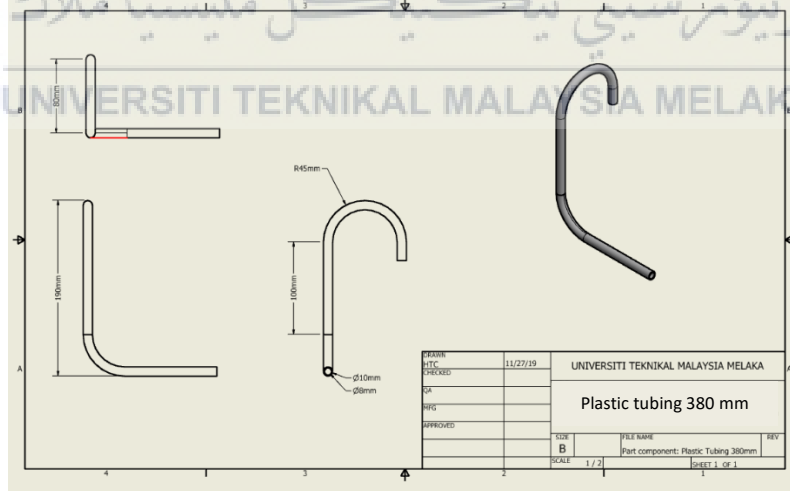
**Figure 4.18:** Plastic tubing 280 mm

Figure 4.19 below shows the plastic tubing that is connected to the on off valve. It has a diameter of 10 mm and 180 mm height.



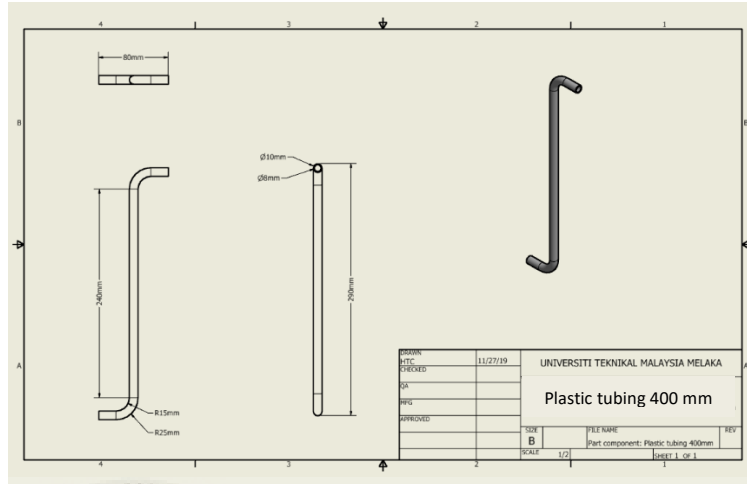
**Figure 4.19:** Plastic tubing 300 mm

Figure 4.20 below shows the plastic tubing that is connected to the biogas reactor and T valve. It has a 10 mm diameter and 190 mm height.



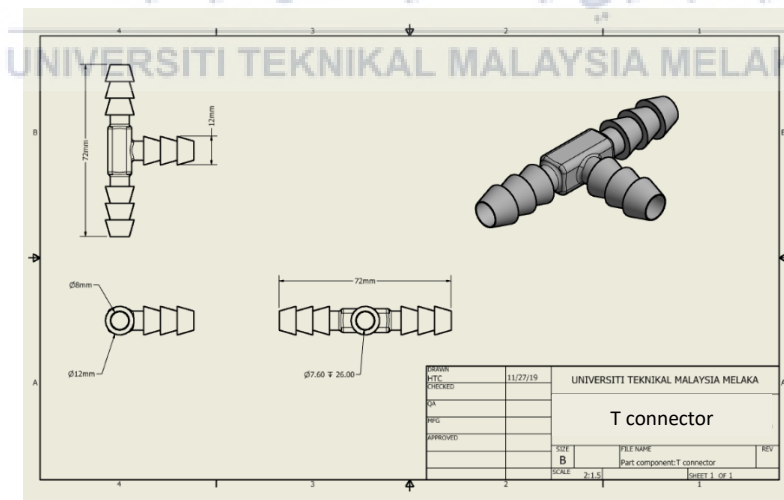
**Figure 4.20:** Plastic tubing 380 mm

Figure 4.21 below shows the plastic tubing that used to transfer the collected gas into the tyre tube. It has a 10 mm diameter and 290 mm height.



**Figure 4.21:** Plastic tubing 400 mm

Figure 4.22 below shows the design of the T connector of the product. The T connector is made up of copper material. It is used to connect the plastic tubing of 280 mm, plastic tubing of 300 mm and plastic tubing of 380 mm. The dimension for the T connector is 72 mm wide, 72 mm in length and the thickness is 12 mm.



**Figure 4.22:** T connector

Figure 4.23 shows the tyre tube that function as a gas storage tank. The tyre tube is made of butyl rubber and is connected to the plastic tubing of 400 mm. It has a length of 492 mm. The outer diameter of the bearing is 492 mm and the inner diameter is 308 mm.

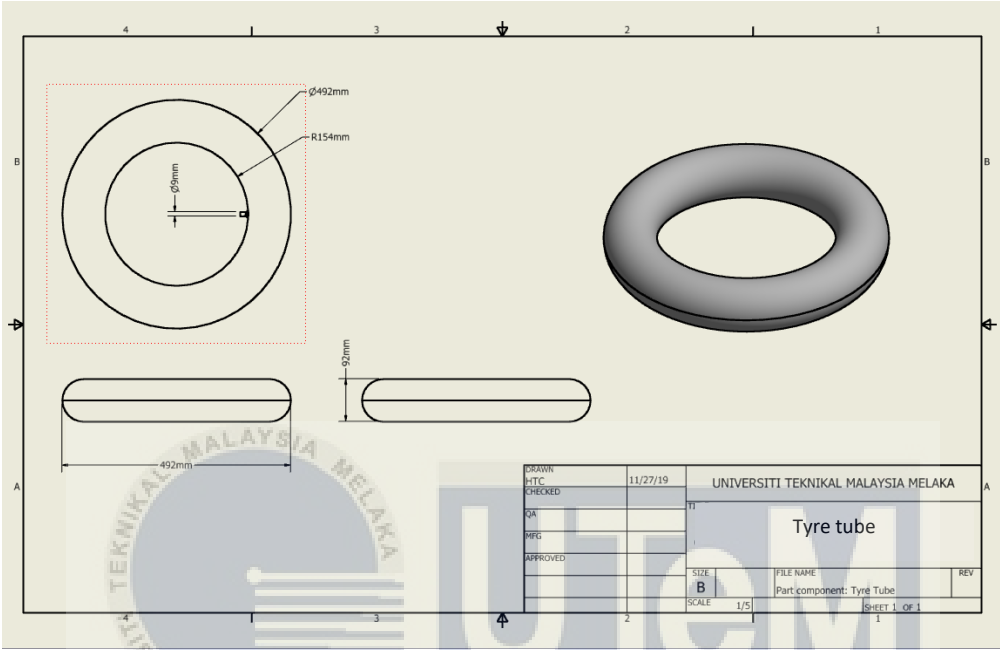


Figure 4.23: Tyre tube

اونيورسيتي تيكنيكل مليسيا ملاك  
UNIVERSITI TEKNIKAL MALAYSIA MELAKA

#### **4.3.6 Materials and components required**

The materials that were used to construct the biogas reactor are listed. Plastic container at a length of 42 cm with the volume capacity of 25 litres is use as the body of biogas reactor, 80 mm PVC (Polyvinyl chloride) pipe as inlet pipe, 210 mm PVC (Polyvinyl chloride) pipe as outlet pipe, 280 mm, 380 mm and 400 mm plastic tubing as connection for gas collection system, 300 mm plastic tubing as gas delivery system, funnel as import tool for slurry, T-connector to connect plastic tubing (380 mm, 280 mm,400 mm) and plastic tubing 300mm and medium size tyre tube as gas storage medium. Superglue, fine sand, soldering iron, blade, and knife were utilized during the fabrication the biogas reactor. As well as, commercial food waste (chicken leftover and rice leftover), cow dung inoculum and tap water.

#### **4.3.7 Fabrication Process of Biogas Mini-Reactor**

Experimental setup included a plastic container as biogas reactor with a capacity of 25 litres. For feeding the industrial food waste and inoculums, PVC pipes will be used, a guide pipe fixed with the biogas reactor chamber and a pipe will be used as the digested slurry outlet. Then, a smaller hole for the gas distribution system, a hole for feeding the slurry at inlet pipe and a hole for replacing the slurry at outlet pipe are made up of three holes. These holes are made with the aid of iron and blade soldering. In the upper half of the bottle, two holes are cut through the diametrically left side of the cylindrical body (one hole as connector for plastic tubing and another hole for inlet tube). In the middle of the bottle, one hole is cut through the right side of the cylindrical body as outlet pipe. Then, with the aid of fine sand and super glue, PVC pipes are attached to the hole.

Slurry consisting of water, industrial food waste (chicken leftover and rice leftover) and cow dung inoculum are loaded in the biogas reactor. To achieve anaerobic conditions, care was taken to ensure zero air entry into the digester. Both the inlet and outlets are closed with pipe plug after the filling of slurry. There are total ten days for cow dung fermentation and ten days for anaerobic digestion. The expansion of the tyre tube occurred within the first three days of the anaerobic digestion cycle due to the output of gas. The contents of the tyre tube will be obtained after the end of the twenty-day period.



#### 4.4 GC-TCD Gas Analysis

Gas Chromatography-Thermal Conductivity Detector (GC-TCD) is a precise method for analysing presence of inorganic gases (argon, nitrogen, hydrogen, carbon dioxide, and so on) and tiny hydrocarbon molecules. The thermal conductivity of two gas flows, which was the pure carrier (reference) gas, and the sample is compared using the GC-TCD. In this case, Argon gases as pure carrier gas and three samples, Chicken to rice ratio (1:1), Chicken to rice ratio (1:2) and Chicken to rice ratio (2:1) are being analyse using GC-TCD. This analysis was performed in the material characterization lab of University Putra Malaysia (UPM).

For output detection analysis, the X-axis is retention time in the unit of minutes and is defined as the time taken (number of minutes) that a compound has spent since it was gone into the Gas Chromatography (GC) injector until it actually hits the thermal conductivity detector. The times at which each of the components arrived to the detector are shown by the peaks. The retention time was greatly influenced by the kind of column used in the analysis, as well as the GC parameters. For instance, flow rate, injection temperature and oven temperature.

Y-axis is known as the area of the peak, which also represents the quantity of particular analyte present. The area for Gas Chromatography-Thermal Conductivity Detector (GC-TCD) was determined by the number of counts collected by the mass spectrometer detector at the retention point.

##### 4.4.1 GC-TCD results

Three samples in this experiment, Chicken to rice ratio (1:1), Chicken to rice ratio (1:2) and Chicken to rice sample (2:1) is being analyse using GC-TCD. The results are generated in graph form and is calibrate using one point calibration method that provided by University Putra Malaysia (UPM) Material Characterization Lab staff. Figure 4.24 shown the GC-TCD gas analysis result of the first sample, which is Chicken to rice ratio (1:1) and followed by Table 4.9 which present the information of output graph as shown in Figure 4.24. For instance, the

retention time, area of the peak, percentage of the total area of the peak, gas detected and ranking of the gas detected. Next, the figure, table and details information of second sample (Chicken to rice ratio (1:2)) and third sample (Chicken to rice ratio (2:1)) were being discussed in the following section.

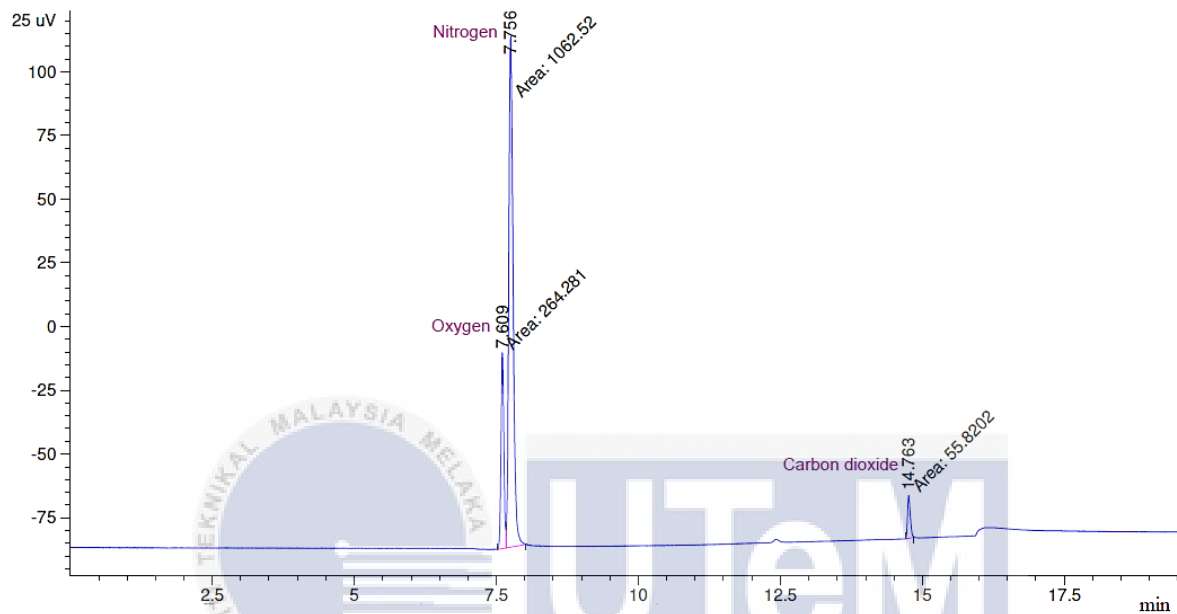
**i. Chicken to rice ratio 1:1**

Referring to Figure 4.24, it shows the gas analysis for chicken to rice (1:1) sample. Also, the gas detection result for control sample in this study as chicken to rice (1:1) sample have equal portion between rice leftover waste and chicken leftover waste and fixed content of cow dung inoculum. At the area of retention for 7.609 minutes, oxygen gas has detected. Based on the one-point calibration Table 4.10, oxygen gas is located at the area of retention at approximately 7.6 minutes. Thus, in this case, oxygen gas is being detected fastest, which is in the retention time of 7.609 minutes. The area of that particular retention time is 264.28082 out of the total area of 1382.62368. The total percentage of that area where oxygen is located is 19.11444%, which rank the second for the number of gases detected.

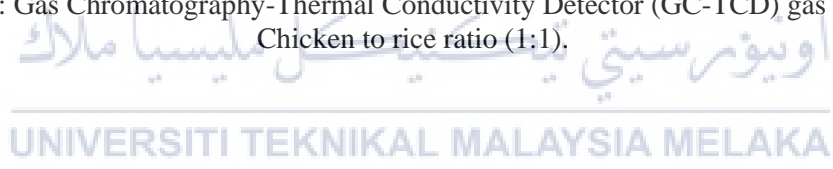
For nitrogen gas, it was detected at the area of retention for 7.756 minutes. Nitrogen gas is located at the area of retention at approximately of 7.7 minutes in the one-point calibration table. In this case, nitrogen was being detected as the second fastest gas after the oxygen gas, which was at the retention time of 7.756 minutes. The area of that particular retention time is 1062.52271 out of the total area of 1382.62368. The total percentage of that area where nitrogen that was located as 76.84829%, which rank as the most detected gas by the Gas Chromatography-Thermal Conductivity Detector (GC-TCD). This indicated the number of counts of nitrogen gases collected by the thermal conductivity detector is the highest at that particular retention point.

On the other hand, carbon dioxide is being detected at the area of retention for 14.763 minutes. Based on one-point calibration table, carbon dioxide gas is located at the area of retention at approximately of 14.7 minutes. Therefore, carbon dioxide gas was considered as the slowest gas that are being detected by GC-TCD. Carbon dioxide gas only can be detected at the retention time of 14.763 minutes. The area of that particular retention time is 55.82015 out of the total area of 1382.62368. The total percentage of that area where carbon dioxide is

located is 4.03726%, which rank the third after nitrogen gases and oxygen gases for the number of gases detected.



**Figure 4.24:** Gas Chromatography-Thermal Conductivity Detector (GC-TCD) gas analysis for Chicken to rice ratio (1:1).



**Table 4.9:** Basic information for the graph analyse in Figure 4.24.

| Retention Time (Min) | $(\frac{Area}{Total Area}) * 100\%$ | Total Area (%) | Gas Detected   | Rank |
|----------------------|-------------------------------------|----------------|----------------|------|
| 7.609                | $\frac{264.28082}{1382.62368}$      | 19.11444       | Oxygen         | 2    |
| 7.756                | $\frac{1062.52271}{1382.62368}$     | 76.84829       | Nitrogen       | 1    |
| 14.763               | $\frac{55.82015}{1382.62368}$       | 4.03726        | Carbon Dioxide | 3    |

**Table 4.10:** One-point calibration for oxygen gas, nitrogen gas and carbon dioxide gas.

| Type of Gas    | Retention Time (min) |
|----------------|----------------------|
| Oxygen         | 7.6                  |
| Nitrogen       | 7.7                  |
| Carbon Dioxide | 14.7                 |

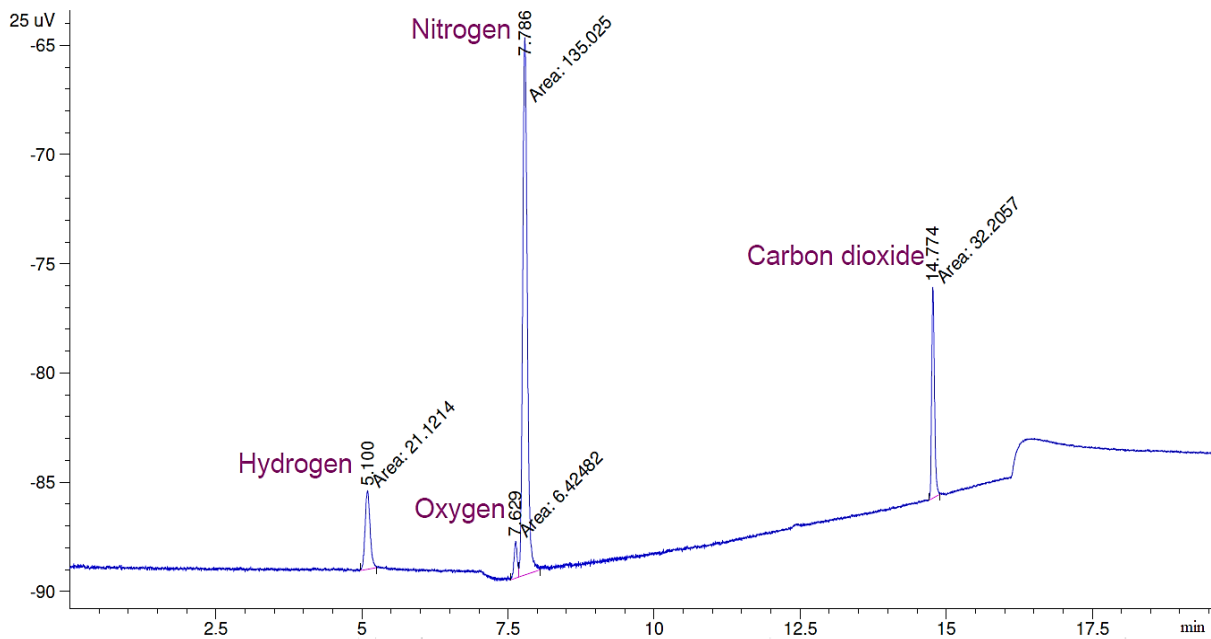
ii. **Chicken to rice ratio 1:2**

Figure 4.25 show the GC-TCD gas analysis for chicken to rice (1:2) sample. The hydrogen gases are considered as the fastest gas release that have been detected by thermal conductivity detector. This can be seen at the area of retention for 5.100 minutes that the hydrogen gases are the gases that appear the fastest compared than other gases. When look into one-point calibration table as shown in Table 4.12, hydrogen gases would appear at the area of retention for 5.0 minutes. Thus, in this case, at the area of retention for 5.100 minutes, it was believing the gases appear is the hydrogen gases. The area of the peak of that particular retention time is 21.1242 out of 194.77741. For total percentages for that particular area, hydrogen gases scored about 10.84388 %. The ranking of hydrogen gases is third where depends on the quantity of particular analyte present in the area of the retention for 5.100 minutes.

At the area of retention for 7.629 minutes, oxygen gas has detected. Based on the one-point calibration table, oxygen gas is located at the area of retention at approximately 7.6 minutes. Thus, in this case, oxygen gas is being detected fastest, which is in the retention time of 7.629 minutes. The area of that particular retention time is 6.42482 out of the total area of 194.77741. The total percentage of that area where oxygen is located is 3.29854%, which rank fourth for the number of gases detected.

For nitrogen gas, it was being detected at the area of retention for 7.786 minutes. Nitrogen gas was located at the area of retention of approximately at 7.7 minutes in the one-point calibration table. In this case, nitrogen was being detected as the second fastest gas after the oxygen gas, which was at the retention time of 7.786 minutes. The area of that particular retention time is 135.02547 out of the total area of 194.77741. The total percentage of that area where nitrogen is located is 69.32296%, which rank the first as the most gases detected by the Gas Chromatography-Thermal Conductivity Detector (GC-TCD).

On the other hand, carbon dioxide is being detected at the area of retention for 14.774 minutes. Based on the one-point calibration table, carbon dioxide gas is located at the area of retention at approximately 14.7 minutes. Therefore, carbon dioxide gas is considered as the slowest gas that are being detected by GC-TCD. Carbon dioxide gas only can be detected at the retention time of 14.774 minutes. The area of that particular retention time is 32.20571 out of the total area of 194.77741. The total percentage of that area where carbon dioxide is located is 16.53462%, which rank the second after nitrogen gases for the number of gases detected.



**Figure 4.25:** Gas Chromatography-Thermal Conductivity Detector (GC-TCD) gas analysis for Chicken to rice ratio (1:2).

**Table 4.11:** Basic information for the graph analyse in Figure 4.25.

| Retention Time (Min) | $\left(\frac{\text{Area}}{\text{Total Area}}\right) * 100\%$ | Total Area (%) | Gas Detected   | Rank |
|----------------------|--|----------------|----------------|------|
| 5.100                | $\frac{21.12142}{194.77741}$                                 | 10.84388       | Hydrogen       | 3    |
| 7.629                | $\frac{6.42482}{194.77741}$                                  | 3.29854        | Oxygen         | 4    |
| 7.786                | $\frac{135.02547}{194.77741}$                                | 69.32296       | Nitrogen       | 1    |
| 14.774               | $\frac{32.20571}{194.77741}$                                 | 16.53462       | Carbon Dioxide | 2    |

**Table 4.12:** One-point calibration for hydrogen gas, oxygen gas, nitrogen gas and carbon dioxide gas

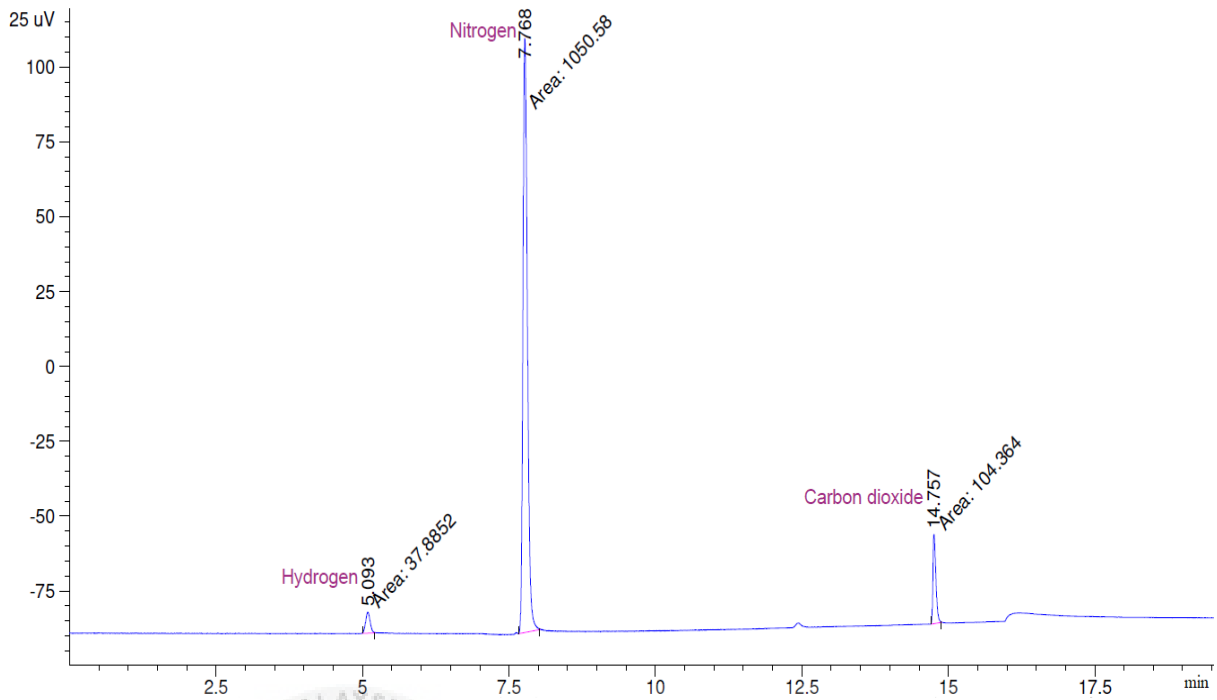
| Type of Gas    | Retention Time (min) |
|----------------|----------------------|
| Hydrogen       | 5.0                  |
| Oxygen         | 7.6                  |
| Nitrogen       | 7.7                  |
| Carbon Dioxide | 14.7                 |

### iii. Chicken to rice ratio 2:1

Figure 4.26 shows the GC-TCD gas analysis for chicken to rice (2:1) sample. The hydrogen gas has detected at the area of retention for 5.093 minutes. Based on one-point calibration table as shown in Table 4.14, hydrogen gas is located at the area of retention at approximately of 5.0 minutes. Thus, in this case, hydrogen gas was being detected fastest, with the retention time of 5.093 minutes. The area of particular retention time is 37.88520 out of the total area of 1192.83078. The total percentage of that area where oxygen is located is 3.17608%, which rank the third after nitrogen gases and carbon dioxide gases for the number of gases detected as shown in Table 4.13.

For nitrogen gas, it was being detected at the area of retention for 7.768 minutes as shown in Figure 4.26. Nitrogen gas was located at the area of retention at approximately of 7.7 minutes in one-point calibration table. In this case, nitrogen was being detected as the second fastest gas after the oxygen gas, which is at the retention time of 7.768 minutes. The area of that particular retention time is 1050.58167 out of the total area of 1192.83078. The total percentage of that area where nitrogen is located is 88.07466%, which ranked first as the most detected gases by Gas Chromatography-Thermal Conductivity Detector (GC-TCD). This has indicated the number of counts for nitrogen gases which collected by the thermal conductivity detector as the highest at that particular retention point.

On the other hand, carbon dioxide is being detected at the area of retention for 14.757 minutes. Based on one-point calibration table, carbon dioxide gas was located at the area of retention at approximately 14.7 minutes. Therefore, carbon dioxide gas is considered as the slowest gas that are being detected by GC-TCD. Carbon dioxide gas only can be detected at the retention time of 14.757 minutes. The area of that particular retention time is 104.36391 out of the total area of 1192.83078. The total percentage of that area where carbon dioxide is located is 8.74926%, which rank the second after nitrogen gases for the number of gases detected.



**Figure 4.26:** Gas Chromatography-Thermal Conductivity Detector (GC-TCD) gas analysis for Chicken to rice ratio (2:1).

**Table 4.13:** Basic information for the graph analyse in Figure 4.26.

| Retention Time (Min) | $\left(\frac{\text{Area}}{\text{Total Area}}\right) * 100\%$ | Total Area (%) | Gas Detected   | Rank |
|----------------------|--|----------------|----------------|------|
| 5.093                | $\frac{37.88520}{1192.83078}$                                | 3.17608        | Hydrogen       | 3    |
| 7.768                | $\frac{1050.58167}{1192.83078}$                              | 88.07466       | Nitrogen       | 1    |
| 14.757               | $\frac{104.36391}{1192.83078}$                               | 8.74926        | Carbon Dioxide | 2    |



**Table 4.14:** One-point calibration for oxygen gas, nitrogen gas and carbon dioxide gas.

| Type of Gas    | Retention Time (min) |
|----------------|----------------------|
| Hydrogen       | 5.0                  |
| Nitrogen       | 7.7                  |
| Carbon Dioxide | 14.7                 |

**Table 4.15:** Presence of gases

| Sample<br>(Chicken to Rice ratio) | Hydrogen<br>(H <sub>2</sub> ) | Nitrogen<br>(N <sub>2</sub> ) | Oxygen<br>(O <sub>2</sub> ) | Carbon dioxide<br>(CO <sub>2</sub> ) | Methane<br>(CH <sub>4</sub> ) |
|-----------------------------------|-------------------------------|-------------------------------|-----------------------------|--------------------------------------|-------------------------------|
| 1:1                               | ✗                             | ✓                             | ✓                           | ✓                                    | ✗                             |
| 1:2                               | ✓                             | ✓                             | ✓                           | ✓                                    | ✗                             |
| 2:1                               | ✓                             | ✓                             | ✗                           | ✓                                    | ✗                             |

The GC-TCD equipment identified many kinds of gases in three of the samples. As indicated in Table 4.15, three gases were discovered in the first sample (Chicken to rice ratio 1:1), which also served as the control sample in this study: nitrogen gases, oxygen gases, and carbon dioxide gases. From Table 4.15, there was no hydrogen present in the chicken-to-rice ratio sample (1:1). Since the total input weight for the chicken to rice (1:1) sample was only 369 grams, the input weight for the ratio of one for chicken leftover waste and one for rice leftover waste is insufficient to produce hydrogen. The total input weight of 553.5 grams for the chicken to rice (1:2) sample and the chicken to rice (2:1) sample, on the other hand, could produce biohydrogen gases. As a result, for future research, the total input weight for the chicken to rice (1:1) sample would be 553.5 grams rather than 369 grams, as the biohydrogen gases may produce in the chicken to rice (1:1) sample. The second sample, on the other hand, contains four kinds of gases: hydrogen gases, nitrogen gases, oxygen gases, and carbon dioxide gases (chicken to rice ratio of 1:2).

The quantity of gases contained in the third sample (Chicken to rice ratio of 2:1) is the same as in the first sample, which contains three kinds of gases. The kinds of gases present, however, vary from those found in the initial sample, which included hydrogen gases, nitrogen gases, and carbon dioxide gases. We can observe from this that nitrogen and carbon dioxide gases are present in all of the samples. According to Anahita Rabii (2019), nitrogen is needed in the feedstock for the synthesis of amino acids, proteins, and nucleic acids. It is also required for the synthesis of ammonia, which is needed to neutralise volatile fatty acids (VFAs) produced during fermentation. Fermentation and the breakdown of organic molecules, on the other hand, are the main producers of carbon dioxide (Ayandotun B. Wasiu, 2012). In this instance, organic substances such as chicken leftover waste and rice leftover waste are fermented and converted into carbon dioxide (CO<sub>2</sub>), a gas composed of two elements: carbon and oxygen.

Thus, in this experiment, the presence of nitrogen gases and carbon dioxide in all samples is deemed normal. Methane, on the other hand, was not present in this experiment. This might be due to the short retention time during cow dung fermentation and anaerobic digestion. In addition, owing to mobility restrictions during this pandemic season, our trial time has been restricted. According to G. Zeeman (2003), methane generation requires a retention period of at least 50 days. Methanogenesis will not occur if this condition is not fulfilled, and the reactor was acidified.

**Table 4.16:** The retention time of gases in minutes

| Sample<br>(Chicken to Rice ratio) | Hydrogen<br>(H <sub>2</sub> ) | Nitrogen<br>(N <sub>2</sub> ) | Oxygen<br>(O <sub>2</sub> ) | Carbon dioxide<br>(CO <sub>2</sub> ) | Methane<br>(CH <sub>4</sub> ) |
|-----------------------------------|-------------------------------|-------------------------------|-----------------------------|--------------------------------------|-------------------------------|
| 1:1                               | -                             | 7.756                         | 7.609                       | 14.763                               | -                             |
| 1:2                               | 5.100                         | 7.786                         | 7.629                       | 14.774                               | -                             |
| 2:1                               | 5.093                         | 7.768                         | -                           | 14.757                               | -                             |

**Table 4.17:** One-point calibration for hydrogen gases, oxygen gas, nitrogen gas and carbon dioxide gas

| Type of Gas    | Retention Time (min) |
|----------------|----------------------|
| Hydrogen       | 5.0                  |
| Oxygen         | 7.6                  |
| Nitrogen       | 7.7                  |
| Carbon Dioxide | 14.7                 |

Retention time can best define as the time taken (number of minutes) that a compound has spent since it was gone into the Gas Chromatography (GC) injector until it actually hits the thermal conductivity detector. From Table 4., the retention time of several type of gases in the form of minutes, each of the gases is fall into the range of their categories. For the retention time of hydrogen gases, it falls into the range of 5.0 to 5.1 minutes. On the other hand, the retention time of nitrogen gases is range between 7.75 to 7.79 minutes. The oxygen gases have the same integer as nitrogen gases, which is seven.

However, the decimal for both of them is different. This can be seen when nitrogen gases are range between 7.75 to 7.79 minutes while oxygen gases are range between 7.60 to 7.63 minutes. For nitrogen gases, the retention time is considered higher compared to the other gases, which is in the range of 14.75 to 14.77 minutes. All of the gases above are being judged due to their retention time that appear in the GC-TCD gas analysis and is calibrated based on the one-point calibration shown in Table 4.17. For instance, the retention time of 5.100 minutes and 5.093 minutes is quite close to the retention time of 5.0 minutes in one-point calibration as shown in Table 4.17. Thus, the gases that located at the retention time of 5.100 minutes and 5.093 minutes is believed as hydrogen gases.

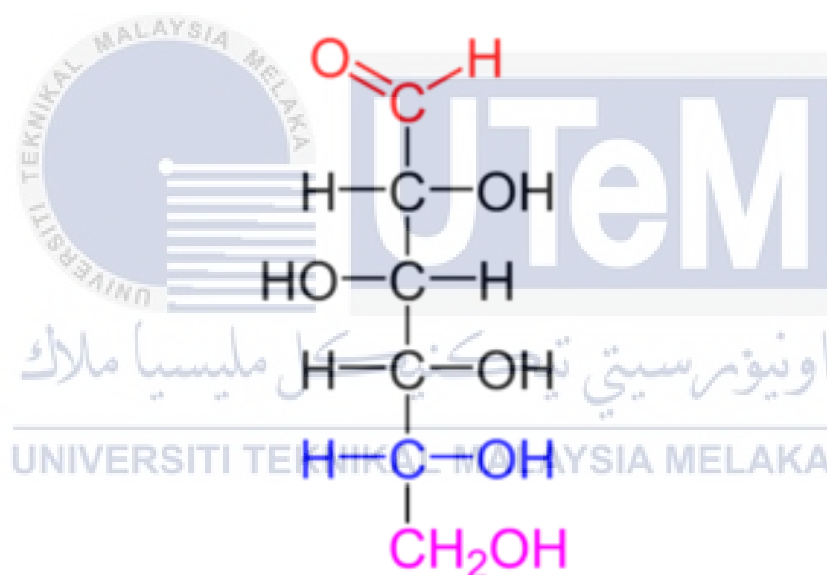
**Table 4.18:** The area of the peaks for gases detection in percentage (%).

| Sample<br>(Chicken to Rice ratio) | Hydrogen<br>(H <sub>2</sub> ) | Nitrogen<br>(N <sub>2</sub> ) | Oxygen<br>(O <sub>2</sub> ) | Carbon dioxide<br>(CO <sub>2</sub> ) | Methane<br>(CH <sub>4</sub> ) |
|-----------------------------------|-------------------------------|-------------------------------|-----------------------------|--------------------------------------|-------------------------------|
| 1:1                               | -                             | 76.84829                      | 19.11444                    | 4.03726                              | -                             |
| 1:2                               | 10.84388                      | 69.32296                      | 3.29854                     | 16.53462                             | -                             |
| 2:1                               | 3.17608                       | 88.07466                      | -                           | 8.74926                              | -                             |

The area of a peak in GC-TCD gas analysis is proportional to the amount of the compound that is present. In this case, there are several areas of peaks that formed by hydrogen gases, nitrogen gases, oxygen gases and carbon dioxide gases. For the first sample (Chicken to rice ratio 1:1), there are three area of peaks is formed. These areas of peaks are nitrogen gases, oxygen gases and carbon dioxide gases. Nitrogen gases have the largest area of peaks, which gather up the total area of peaks up to 76.84829% out of 100%. This indicated that the amount of nitrogen gases that present in the first sample (Chicken to rice ratio 1:1) is relatively high. And this is followed by the oxygen gases that have 19.11444% and the carbon dioxide gases of 4.03726%.

For second sample (Chicken to rice ratio 1:2), we can see there are four area of peaks as shown in Table 4.18. These areas of peaks are hydrogen gases, nitrogen gases, oxygen gases and carbon dioxide gases. Nitrogen gases still have the largest area of peaks, which gather up the total area of peaks up to 69.32296 % out of 100%. And followed by the second highest area of peak, carbon dioxide gases that have 16.53462% of its compound in the second sample (Chicken to rice ratio 1:2). Both hydrogen gases and oxygen gases have a relatively low content of its compound in the second sample (Chicken to rice ratio 1:2), which is 10.84388% and 3.29854% respectively. For the third sample (Chicken to rice sample 2:1), there are three area of peaks is formed, which is contributed by hydrogen gases, nitrogen gases and carbon dioxide gases. Nitrogen contains the highest percentage for the area of peaks, which is 88.07466% and followed by carbon dioxide gases that contain 8.74926% for the area of peaks. The amount of hydrogen gases in the third sample (Chicken to rice ratio 2:1) is relatively low in which it's percentage for the area of peaks is only 3.17608% out of 100%.

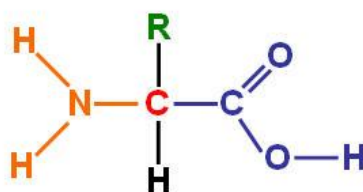
In this study, the biohydrogen gases were detected in the chicken to rice ratio (1:2) sample and the chicken to rice ratio (2:1) sample. Also, the biohydrogen content in the chicken to rice (1:2) sample was found to be higher compared to the other samples. It can be said that the production of biohydrogen in chicken to rice (1:2) sample is basically influenced by the presence of rice leftover waste. This is well supported by the chemical molecular structure of rice leftover waste, which has a higher number of hydrogen (H) atoms in comparison to chicken leftover waste, which has a higher carbon (C) atom than hydrogen (H) atom as shown in Table 4.19. There are a total of twelve hydrogen (H) atoms present in the rice leftover waste, which is almost triple the amount of the chicken leftover waste, which has only three (3) hydrogen (H) atoms.



**Figure 4.27:** Chemical molecular structure for rice waste leftover.

(Sources: (<https://www.alamy.com/protein-structural-chemical-formula-and-molecular-model-general-formula-of-amino-acids-vector-illustration-image370396718.html>) access on: 20 August

2021)



**Figure 4.28:** Chemical molecular structure for chicken waste leftover.

(Sources: (<https://biochemistryquestions.wordpress.com/2009/04/23/classification-of-carbohydrates/>) access on 20 August 2021)

**Table 4.19:** Total number of carbon (C) atom and hydrogen (H) atom for the chicken waste leftover and rice waste leftover sample.

| Type of atom      | Chicken leftover waste | Rice leftover waste |
|-------------------|------------------------|---------------------|
| Carbon (C) atom   | 2                      | 6                   |
| Hydrogen (H) atom | 4                      | 12                  |

#### 4.4.2 FTIR results

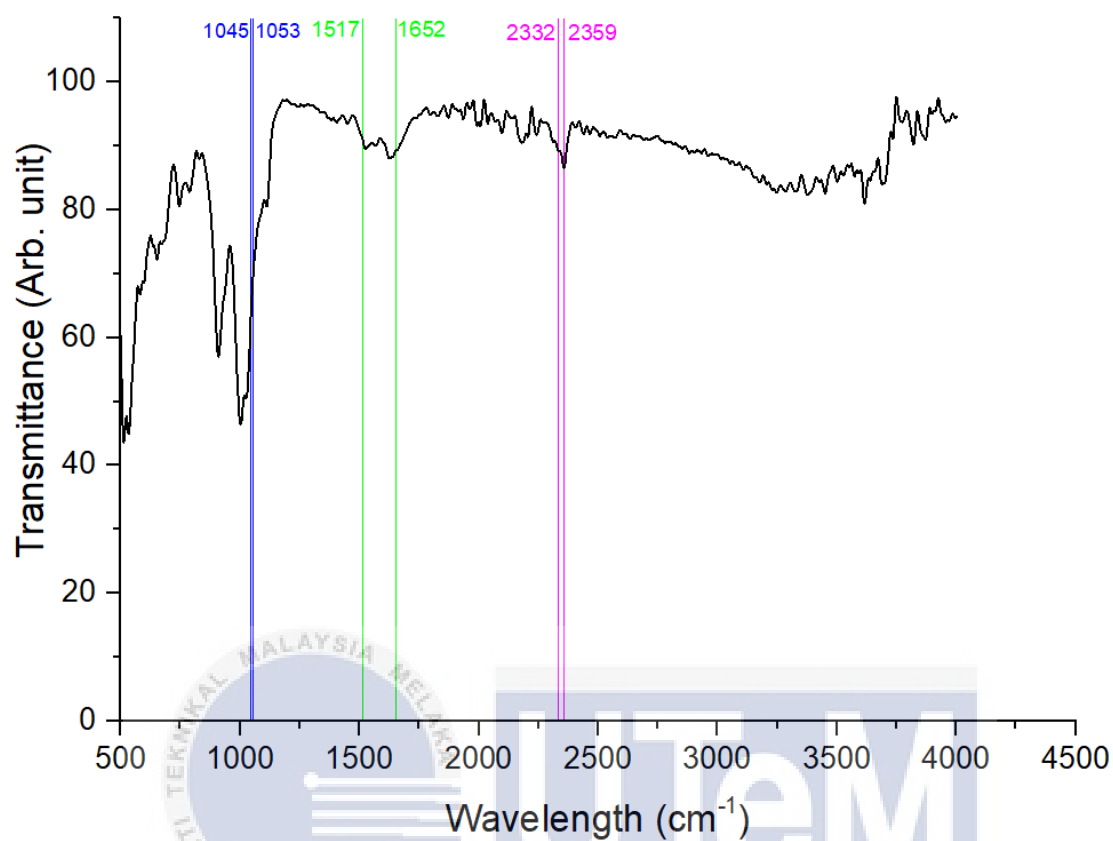
##### i. Chicken to rice ratio (1:1)

Figure 4.29 shows the Fourier-transform Infrared Spectroscopy (FTIR) analysis for control sample in this study, which is chicken to rice ratio (1:1) sample. In this case, the 1:1 ratio represent to the weight of 184.5 grams of chicken waste leftover and 184.5 grams of rice waste leftover. Figure 4.29 shows that the chicken to rice ratio (1:1) sample have comes out with three peaks in FTIR gas analysis result. From those three peaks, there are three different colours lines, which are blue colour, green colour and pink colour. Each colour represents different chemical compound and the wavelength range of the chemical compound. Therefore,

there are total of three chemical component appears in this chicken to rice ratio (1:1) sample. For the blue colour lines that range between the wavelength of  $1045\text{ cm}^{-1}$  to  $1053\text{ cm}^{-1}$ , it indicates that there is the presence of carbohydrates compound. Mordechai (2001) reported that there is C-O stretching coupled with C-O bending of the C-OH of carbohydrates structure inside this carbohydrate component.

On the other hand, there are the presence of protein (amide I) and protein (amide II) structure in the green colour lines that range between the wavelength of  $1517\text{ cm}^{-1}$  to  $1652\text{ cm}^{-1}$ . For the protein (amide I) component, there are presence of C=C uracyl and C=O chemical structure (Schulz and Baranska, 2007). In contrast, there are presence of stretching C=N, C=C and C=N guanine structure in amide (II) component (Paluszkiewicz and Kwiatek, 2001). This indicates that although amide (I) and amide (II) is coming from the same chemical component, their chemical structure can still be totally different from each other.

For the third peak that count from the left, labelled with pink colour lines and in the range at between of  $2332\text{ cm}^{-1}$  to  $2359\text{ cm}^{-1}$ . At this range, there are the presence of amino-related component. According to Nandiyanto et al. (2018b), there are the presence of NH component structure. NH is named as imidogen and is found that it is highly reactive and consequently short-lived.



**Figure 4.29:** Fourier-transform Infrared Spectroscopy (FTIR) analysis for chicken to rice ratio (1:1) sample.

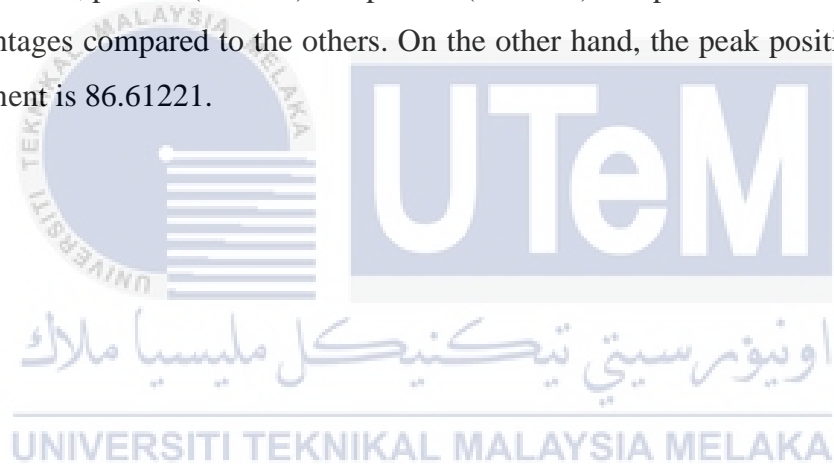
**Table 4.20:** Basic information regarding band assignment for the range that stated in the Figure 4.29.

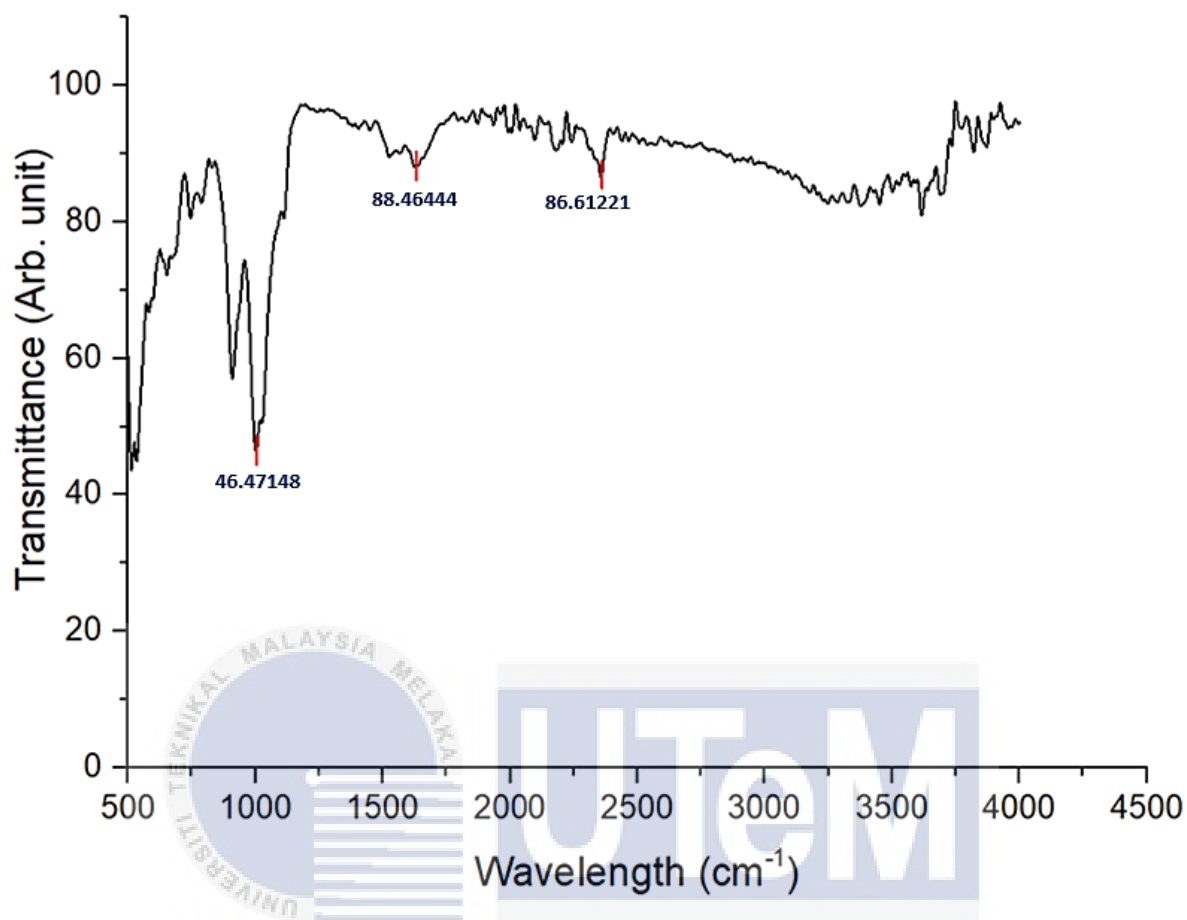
| RANGE     | COMPONENT                                    | REFERENCES   |
|-----------|--|--|
| 1045-1053 | Carbohydrate                                 | Mordechai., 2001   |
| 1517-1652 | Protein (Amide I)<br>&<br>Protein (Amide II) | Schulz and Baranska, 2007<br>Paluszkiewicz and Kwiatek, 2001 |
| 2332-2359 | Amino-related component                      | Nandiyanto, 2018b  |



Figure 4.30 shown the peak position for the chemical component of chicken to rice ratio (1:1) sample that have been discussed in Figure 4.29. There are total of three peaks as shown in Figure 4.30, namely as carbohydrates, protein (amide I), protein (amide II) and amino-related component count from the left. Each of them has a peak position that has labelled with a red line in Figure 4.30. The peak position unit is labelled in according to the transmittance that located at y-axis.

According to Mordechai (2001), for carbohydrates peak, it has 46.47148 for its peak position, which is after protein (amide I) and protein (amide II) peak and amino-related component peak. On the other hand, for the next peak, Schulz and Baranska (2007) reported that protein (amide I) and protein (amide II) has gain 88.46444 for its peak position. This can be seen in Table 4.21, protein (amide I) and protein (amide II) component have the highest peak position percentages compared to the others. On the other hand, the peak position for amino-related component is 86.61221.





**Figure 4.30:** Peak position for carbohydrates, protein (amide I), protein (amide II), fat and water in the chicken to rice ratio (1:1) sample.

**Table 4.21:** Some information regarding the peak position and chemical component in the chicken to rice ratio (1:1) sample.

| PEAK POSITION | COMPONENT                              |
|---------------|--|
| 46.47148      | Carbohydrate                           |
| 88.46444      | Protein (Amide I) & Protein (Amide II) |
| 86.61221      | Amino-related component                |

Figure 4.32 show the peak intensity for carbohydrates, protein (amide I), amide (II) and water component in the chicken to rice ratio (1:2) sample. Peak intensity was considered as the area of concentration for that particular peak. As we can see in Figure 4.32, there are several red cross marks on certain peaks. This red cross mark indicated as the top value of the peak and the bottom value of the peak.

For the first peak that count from the left as shown in Figure 4.32, it is the carbohydrate peak and the top value for its peak position is 81.557 while the bottom value for its peak position is 46.47148. Thus, the difference of these two peak position values have given the total peak intensity value of 35.08552. This indicates that there is high concentration of carbohydrate is detected in this chicken to rice ratio (1:1) sample compared to others chemical content. This might due to the presence of white flour that being used to fried the chicken, which in this case, Kentucky Fried Chicken (KFC) as the substrate. Before taking Kentucky Fried Chicken (KFC) into deep fried, basically on top of the chicken skin layer, there is covered with white flour. Pawan Kumar (2011) stated that there is 77.7 of carbohydrates composition from the white flour. Therefore, the high carbohydrates content of 35.08552 in the chicken to rice ratio (1:1) is make sense due to the addition of white flour in Kentucky Fried Chicken (KFC) as deep-fried purpose. And also, the carbohydrates content in rice leftover waste itself. Figure 4.31 show the rice leftover waste that appear in greyish colour and chicken leftover waste that appear in brownish colour which contain of carbohydrates content.

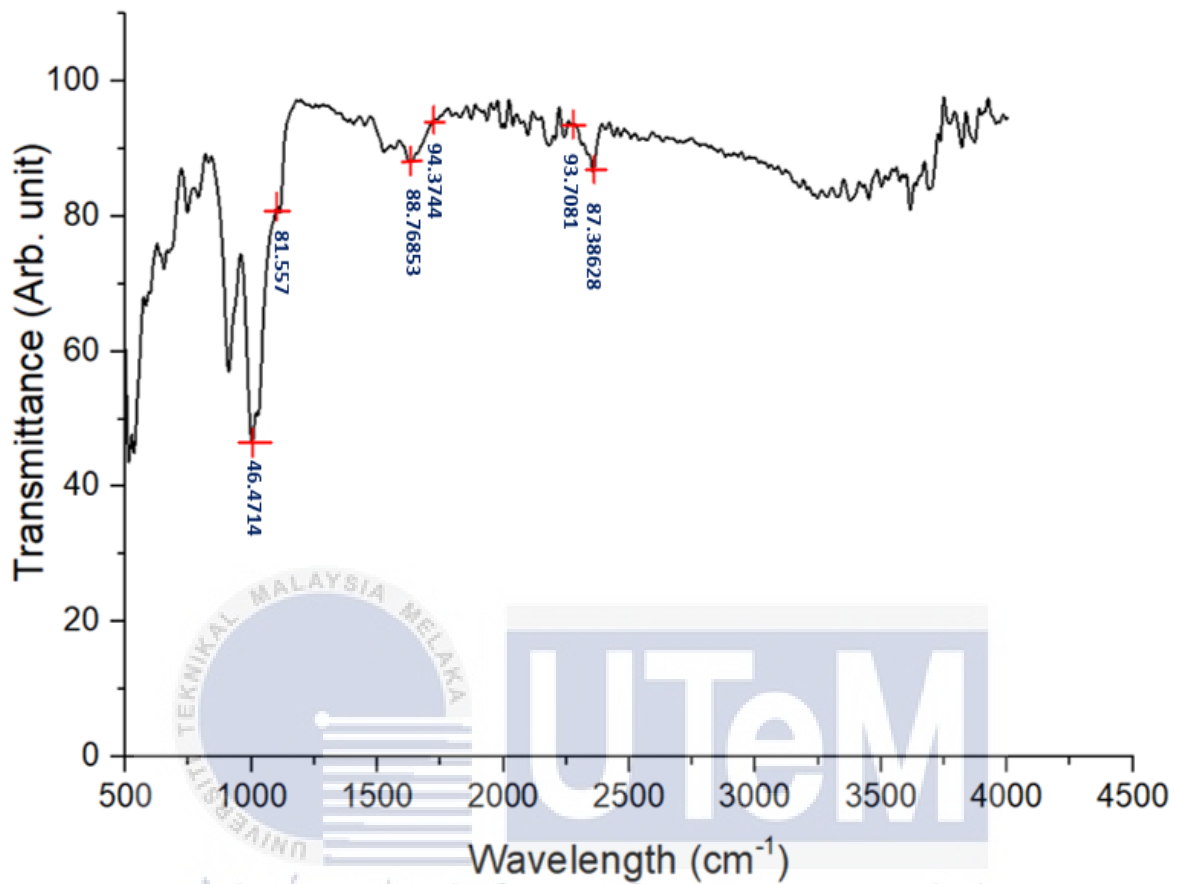


**Figure 4.31:** Dried rice waste leftover (greyish colour), chicken waste leftover (brownish colour) and cow dung inoculum (charcoal black colour) in chicken to rice ratio (1:1) sample.

On the other hand, protein (amide I) and protein (amide II) component which have the total peak intensity value of 5.60587 was ranked at the third place after the carbohydrate peak and amino-related component peak. This is due to the differences between the top peak position value of 94.3744 and bottom peak position value of 88.76853 as shown in Table 4.22. According to Hassan Mohammed (2020), there are approximately 20.02% of protein content in the chicken itself. Therefore, protein (amide I) and protein (amide II) are believed comes from the chicken leftover waste that have the ratio of one which in 184.5 grams. The amount of dried chicken leftover waste is appeared as brownish colour as shown in Figure 4.31.

UNIVERSITI TEKNIKAL MALAYSIA MELAKA

For the third peak that count from the left, is the peak location for amino-related component. For this amino-related component, the top peak position value is 93.7081 and bottom peak position value is 87.38628 as shown in Table 4.22. The differences between these two peak intensity values have given a total peak intensity value of 6.32182. This indicated that there are 6.32182 concentration of the amino-related component. The amino-related component is believed is the formation of amino acid that present in rice itself. According to Abdul Rohman (2014), rice contains essential amino acids for nutrient purpose.



**Figure 4.32:** Peak intensity for carbohydrates, protein (amide I), amide (II) and fat component in the chicken to rice ratio (1:1) sample

**Table 4.22:** Peak intensity information for carbohydrates, protein (amide I), amide (II) and fat component in the chicken to rice ratio (1:1) sample

| PEAK INTENSITY   | TOTAL PEAK INTENSITY | COMPONENT                              |
|------------------|----------------------|--|
| 81.557-46.47148  | 35.08552             | Carbohydrate                           |
| 94.3744-88.76853 | 5.60587              | Protein (Amide I) & Protein (Amide II) |
| 93.7081-87.38628 | 6.32182              | Amino-related component                |

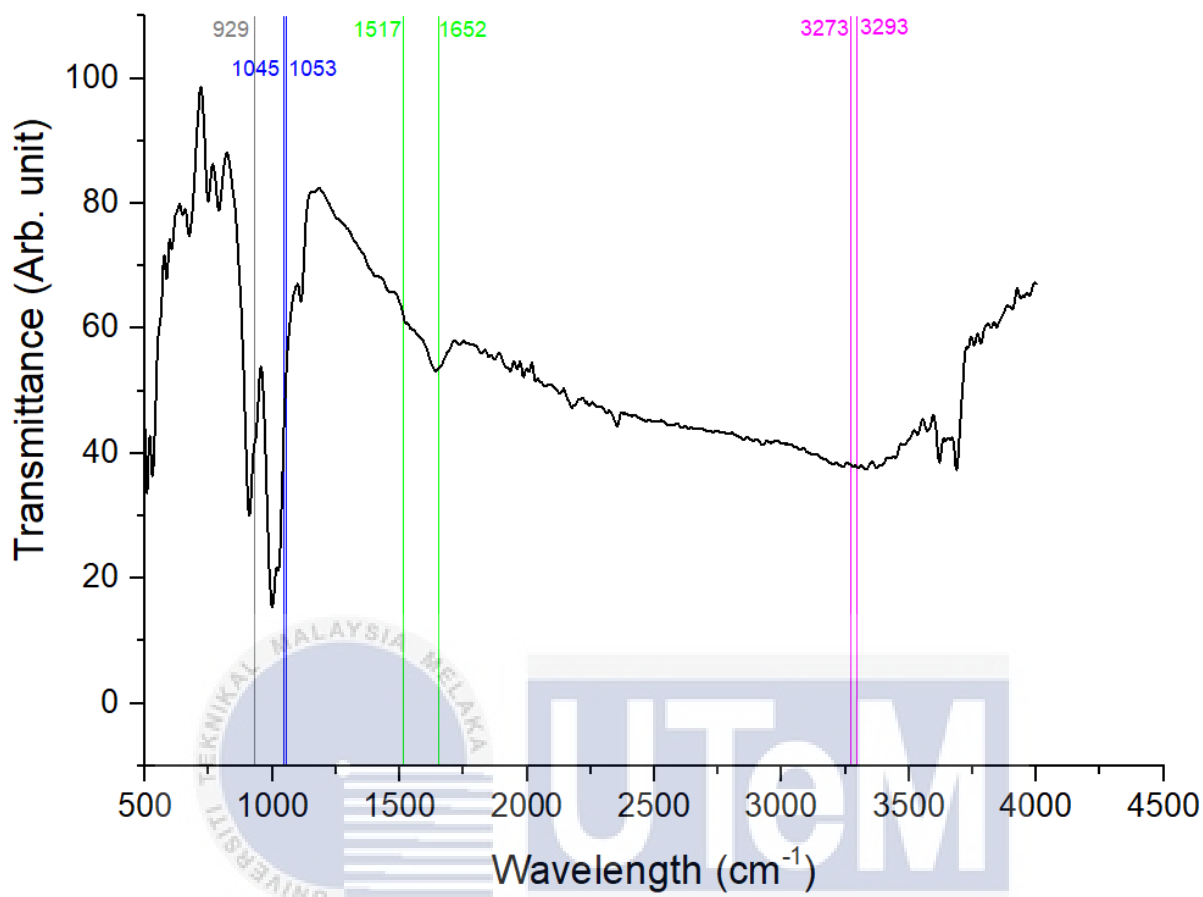
## ii. Chicken to rice ratio (1:2)

Figure 4.33 shows the Fourier-transform Infrared Spectroscopy (FTIR) analysis for chicken to rice ratio (1:2) sample. In this case, the 1:2 ratio represent to the weight of 184.5 grams of chicken leftover waste and 369 grams of rice leftover waste. And from Figure 4.33, we can see that chicken to rice ratio (1:2) sample have comes out of four peaks in the FTIR gas analysis result. From those four peaks, we can see there is four different colours lines, which is grey colour, blue colour, green colour and pink colour. Each colour represents different chemical compound and the wavelength range of the chemical compound. Therefore, there are total of four chemical component appears in this chicken to rice ratio (1:2) sample. For the grey colour lines that range at the wavelength of  $929\text{ cm}^{-1}$ , it indicates that there is the presence of carbon compound. Nandiyanto (2016) stated that there is carbon-related structure inside this carbon component.

At the wavelength of  $1045\text{ cm}^{-1}$  to  $1053\text{ cm}^{-1}$ , it is found that there is the presence of carbohydrates compound which labelled by blue colour lines. Mordechai (2001) reported that there is C-O stretching coupled with C-O bending of the C-OH of carbohydrates structure inside this carbohydrate component.

On the other hand, there are the presence of protein (amide I) and protein (amide II) structure in the green colour lines that range between the wavelength of  $1517\text{ cm}^{-1}$  to  $1652\text{ cm}^{-1}$ . For the protein (amide I) component, there are presence of C=C uracyl and C=O chemical structure (Schulz and Baranska, 2007). In contrast, there are presence of Stretching C=N, C=C and C=N guanine structure in amide (II) component (Paluszkiwicz and Kwiatek, 2001). This indicates that although amide (I) and amide (II) is coming from the same chemical component, their chemical structure can still be totally different from each other.

For the pink colour lines that range between the wavelength of  $3273\text{ cm}^{-1}$  to  $3293\text{ cm}^{-1}$ , we can see that there is the presence of water compound as shown in Table 4.23. Schulz and Baranska (2007) reported that there is stretching O-H symmetric structure inside this water component.



**Figure 4.33:** Fourier-transform Infrared Spectroscopy (FTIR) analysis for chicken to rice ratio (1:2) sample.

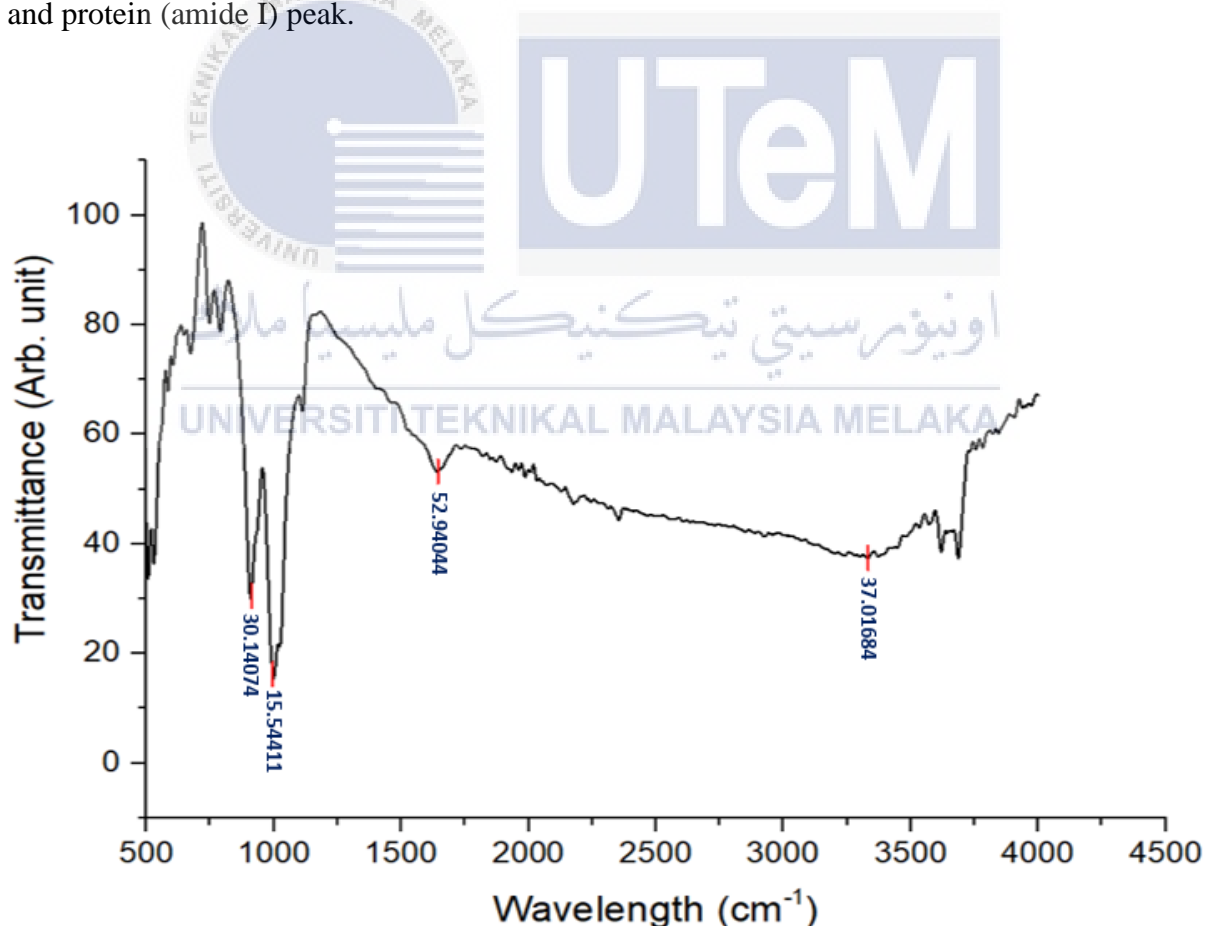
UNIVERSITI TEKNIKAL MALAYSIA MELAKA

**Table 4.23:** Basic information regarding band assignment for the range that stated in the Figure 4.33.

| RANGE     | COMPONENT                                    | REFERENCES  |
|-----------|--|---|
| 929       | Carbon                                       | Nandiyanto, 2016  |
| 1045-1053 | Carbohydrate                                 | Mordechai, 2001   |
| 1517-1652 | Protein (Amide I)<br>&<br>Protein (Amide II) | Schulz and Baranska, 2007<br><br>Anuszkiewicz and Kwiatek, 2001 |
| 3273-3293 | Water  | Schulz and Baranska, 2007                                       |

Figure 4.34 shown the peak position for the chemical component of chicken to rice ratio (1:2) sample that have been discussed in Figure 4.33. There is total four peaks as shown in Figure 4.34, named as carbon, carbohydrates, protein (amide I), protein (amide II) and water that count from the left. Each of them has a peak position that has labelled with a red line in Figure 4.34. The peak position unit is labelled in percentage according to the transmittance that located at y-axis.

For the first peak that located from left, the carbon peak has gain 30.14074 for its peak position. Mordechai (2001) stated that for carbohydrates peak, it has 15.54411 for its peak position. For the next peak, protein (amide I) and protein (amide II) has gain 52.94044 for its peak position. On the other hand, Schulz and Baranska (2007) reported that water peak has the peak position percentages of 37.01684, which rank at the second place after the protein (amide I) and protein (amide II) peak.



**Figure 4.34:** Peak position for carbohydrates, protein (amide I), protein (amide II), fat and water in the chicken to rice ratio (1:2) sample.

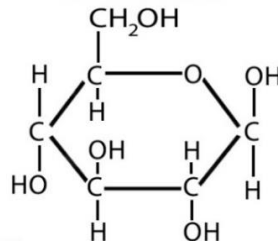


**Table 4.24:** Some information regarding the peak position and chemical component in the chicken to rice ratio (1:2) sample.

| PEAK POSITION | COMPONENT                              |
|---------------|--|
| 30.14074      | Carbon                                 |
| 15.54411      | Carbohydrate                           |
| 52.94044      | Protein (amide I) & Protein (amide II) |
| 37.01684      | Water                                  |

Figure 4.37 show the peak intensity for carbohydrates, protein (amide I), amide (II) and water component in the chicken to rice ratio (1:2) sample. Peak intensity is considered as the area of concentration for that particular peak. As we can see in Figure 4.37, there are several red cross marks on certain peaks. This red cross mark indicated as the top value of the peak and the bottom value of the peak. For the first peak that count from the left as shown in Figure 4.37, it is the carbon peak and the top value for its peak position is 87.65268 while the bottom value for its peak position is 30.98517.

Thus, the difference of these two peak position values have given the total peak intensity value of 56.66751. This indicates that there is high concentration of carbon is detected in this chicken to rice ratio (1:2) sample compared to others. This might due to the presence of glucose from carbohydrates that belongs to rice. For your information, there are consists of several carbon atom inside the molecular structure of carbohydrates. When look into detail in Figure 4.35, we can see there is total of six carbon atoms existed in the glucose structure that belongs to carbohydrates. Mordechai (2001) reported that the quantity of carbon atom will be more than the quantity of carbohydrates itself when there is the increase amount of rice. This is due to carbon is a chain of atoms which are bound together, to form a bigger molecule, which is called a glucose. Hence, for the carbohydrates, glucose as a molecule will bound with another glucose to form carbohydrates.



**Figure 4.35:** Molecular structure of glucose

(Sources: <https://www.nutrientsreview.com/carbs/monosaccharides-glucose.html> , access on: 20 August 2021)

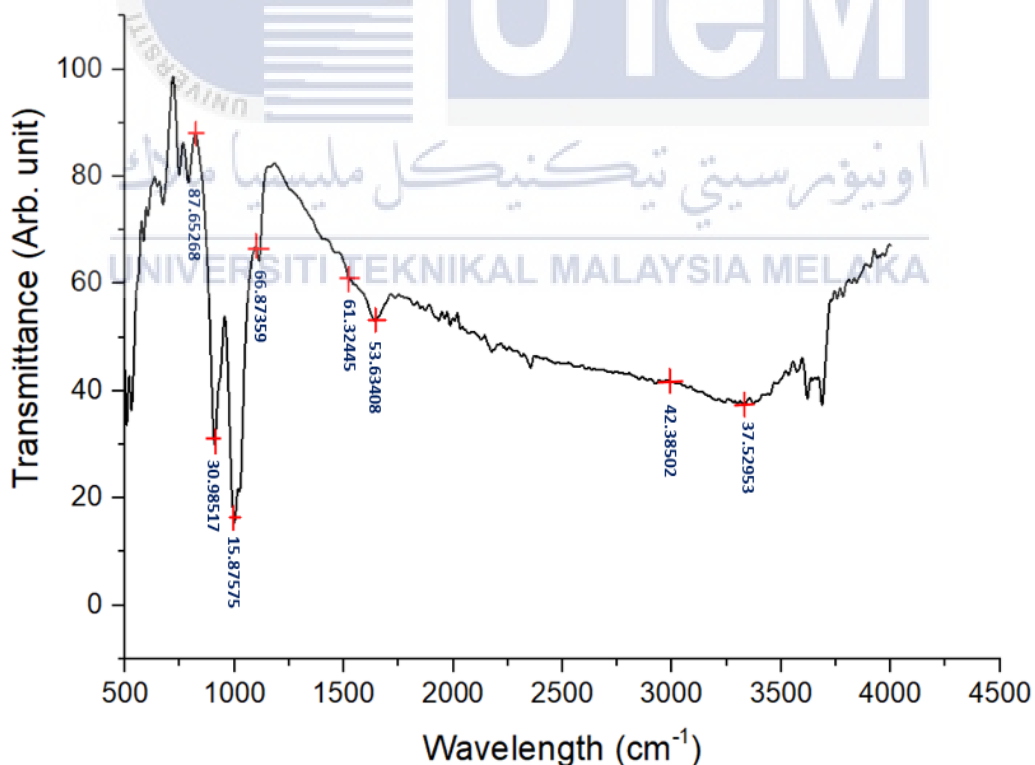
For the carbohydrates peak that located at the second position from the left after the carbon peak, the total peak intensity value is 50.99774. This is due to the differences between the top peak position value of 66.87359 and bottom peak position value of 15.87585. As we can see in Table 4.25, carbohydrate peak rank at the second after carbon peak. This is due to the ratio of rice is two that is doubled than the ratio of chicken, which is one in ratio. This can be seen when there is 369 gram of rice leftover waste and there is only 184.5 grams of chicken leftover waste is inserted into the chicken to rice ratio (1:2) sample. For your information, rice is good sources of carbohydrates. And thus, high amount of rice will consequently produce high amount of carbohydrates. This can be seen in Figure 4.36, the amount of dried rice leftover waste that in yellowish colour is relatively high compared to amount of dried chicken leftover that appeared in brownish colour.



**Figure 4.36:** Dried rice waste leftover (yellowish colour), chicken waste leftover (brownish colour) and cow dung inoculum (charcoal black colour) in chicken to rice ratio (1:2) sample.

In contrast, Schulz and Baranska (2007) stated that protein (amide I) and protein (amide II) component which have the total peak intensity value of 7.69037 is rank at the third. This is due to the differences between the top peak position value of 61.32445 and bottom peak position value of 53.63408 as shown in Table 4.25. This protein (amide I) and protein (amide II) is believed comes from the chicken leftover waste that have the ratio of one which in 184.5 grams. The amount of dried chicken leftover waste is appeared as brownish colour as shown in Figure 4.36.

On the other hand, for the fourth peak that count from the left, is the peak location for water component. For this water component, the top peak position value is 42.38502 and bottom peak position value is 37.52953. as shown in Table 4.25. The differences between these two peak intensity values have given a total peak intensity value of 4.85549 which rank the fourth after carbon, carbohydrate and protein (amide I) and protein (amide II). This indicates that water content in the chicken to rice ratio (1:2) is relatively low compared to others component.



**Figure 4.37:** Peak intensity for carbohydrates, protein (amide I), amide (II) and water component in the chicken to rice ratio (1:2) sample.

**Table 4.25:** Peak intensity information for carbohydrates, protein (amide I), amide (II) and water component in the chicken to rice ratio (1:2) sample.

| PEAK INTENSITY    | TOTAL PEAK INTENSITY | COMPONENT                              |
|-------------------|----------------------|--|
| 87.65268-30.98517 | 56.66751             | Carbon                                 |
| 66.87359-15.87585 | 50.99774             | Carbohydrate                           |
| 61.32445-53.63408 | 7.69037              | Protein (Amide I) & Protein (Amide II) |
| 42.38502-37.52953 | 4.85549              | Water                                  |

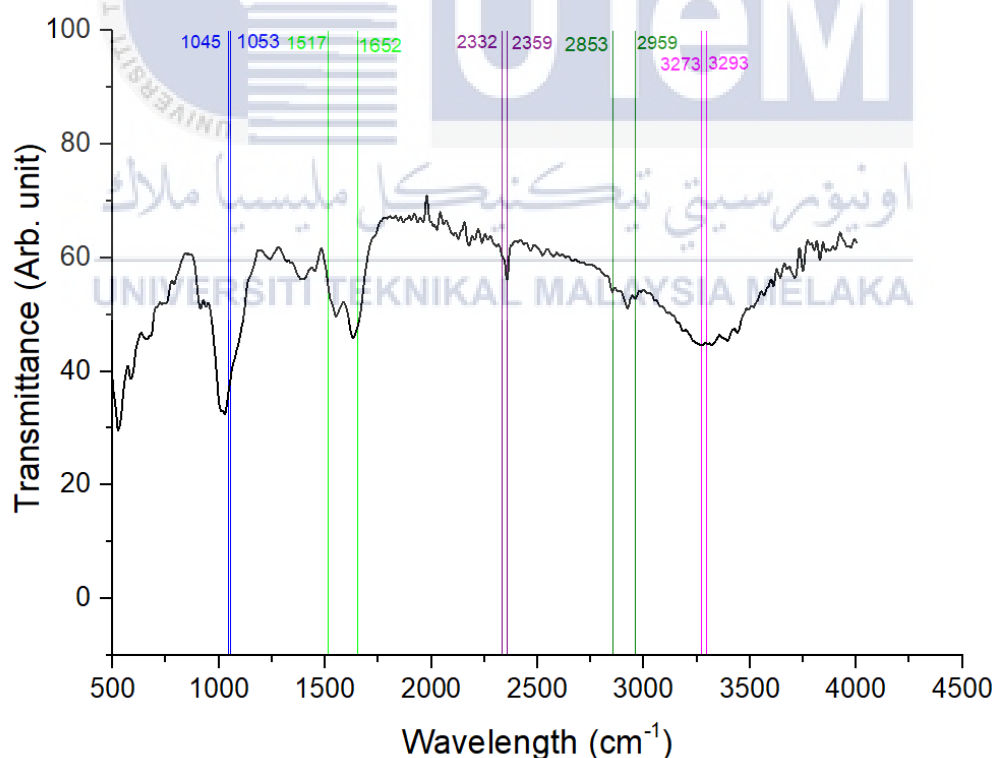
### iii. Chicken to rice ratio (2:1)

Figure 4.38 show the Fourier-transform Infrared Spectroscopy (FTIR) analysis for chicken to rice ratio (2:1) sample. In this case, the 2:1 ratio represent the weight of 369 grams of chicken leftover waste and 184.5 grams of rice leftover waste. And from Figure 4.38, we can see that chicken to rice ratio (2:1) sample have comes out of five peaks in the FTIR gas analysis result. From these five peaks, we can see there is five different colours lines, which is blue colour, light green colour, purple colour, dark green colour and pink colour. Each colour represents different chemical compound and the wavelength range of the chemical compound. Therefore, there are total of five chemical components appears in this chicken to rice ratio (2:1) sample. For the blue colour lines that range between the wavelength of  $1045\text{ cm}^{-1}$  to  $1053\text{ cm}^{-1}$ , it indicates that there is the presence of carbohydrates compound as shown in Table 4.26. Mordechai (2001) reported that there is C-O stretching coupled with C-O bending of the C-OH of carbohydrates structure inside this carbohydrate component.

On the other hand, there are the presence of protein (amide I) and protein (amide II) structure in the light green colour lines that range between the wavelength of  $1517\text{ cm}^{-1}$  to  $1652\text{ cm}^{-1}$ . For the protein (amide I) component, there are presence of C=C uracyl and C=O chemical structure (Schulz and Baranska, 2007). In contrast, there are presence of stretching C=N, C=C and C=N guanine structure in amide (II) component (Paluszkiwicz and Kwiatek, 2001). This

indicates that although amide (I) and amide (II) is coming from the same chemical component, their chemical structure can still be totally different from each other.

For the third peak that count from the left, we can see it is label with purple colour lines and is range from the wavelength of  $2332\text{ cm}^{-1}$  to  $2359\text{ cm}^{-1}$ . At this range, there are the presence of amino-related component. According to Nandiyanto (2018b), there are the presence of NH component structure. NH is named as imidogen and is found that it is highly reactive and consequently short-lived. There is also presence of fat chemical component in the chicken to rice ratio (2:1) sample. This fat chemical component is located in the range of wavelength  $2853\text{ cm}^{-1}$  to  $2959\text{ cm}^{-1}$  that labelled with dark green colour lines. Yang (2005) stated that there is the presence of  $\text{CH}_2$  of lipids structure in this fat component. For the pink colour lines that range between the wavelength of  $3273\text{ cm}^{-1}$  to  $3293\text{ cm}^{-1}$ , it indicates that there is the presence of water compound in the chicken to rice ratio (2:1) sample. Schulz and Baranska (2007) reported that there is existence of stretching O-H symmetric structure in water component.



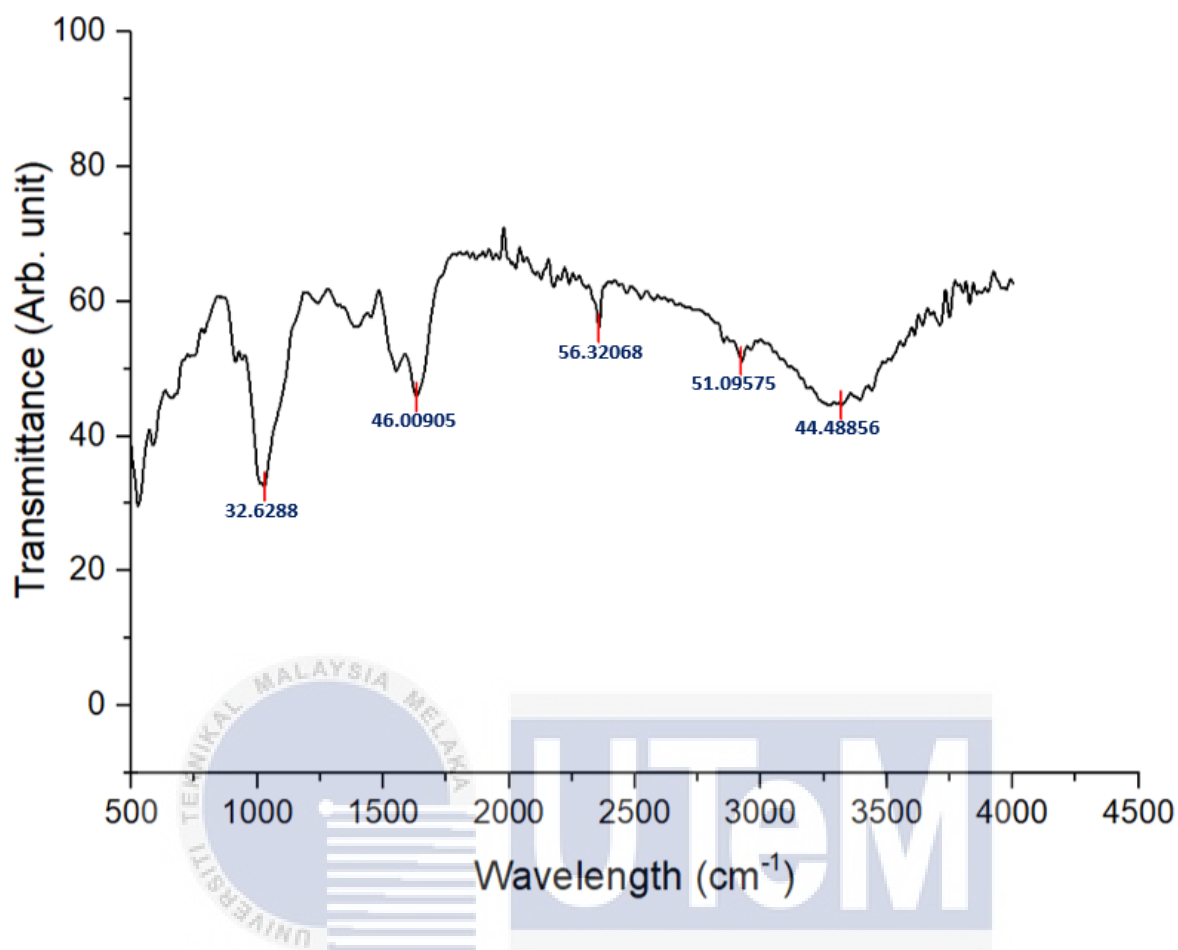
**Figure 4.38:** Fourier-transform Infrared Spectroscopy (FTIR) analysis for chicken to rice ratio (2:1) sample.

**Table 4.26:** Basic information regarding component for the range that stated in the Figure 4.38.

| RANGE     | COMPONENT                                    | REFERENCES   |
|-----------|--|--|
| 1045-1053 | Carbohydrate                                 | Mordechai, 2001  |
| 1517-1652 | Protein (Amide I)<br>&<br>Protein (Amide II) | Schulz and Baranska, 2007<br>Paluszkiewicz and Kwiatek, 2001 |
| 2332-2359 | Amino-related component                      | Nandiyanto, 2018b  |
| 2853-2959 | Fat  | Yang, 2005   |
| 3273-3293 | Water  | Schulz and Baranska, 2007                                    |

Figure 4.39 shown the peak position for the chemical component of chicken to rice ratio (2:1) sample that have been discussed in Figure 4.38. There is total four peaks as shown in Figure, named as carbon, carbohydrates, protein (amide I), protein (amide II) and water that count from the left. Each of them has a peak position that has labelled with a red line in Figure 4.39. The peak position unit is labelled in percentage according to the transmittance that located at y-axis.

For the first peak that located from left, the carbohydrate peak has gain 32.6288 for its peak position as shown in Table 4.27. For protein (amide I) and protein (amide II) peak, it has 46.00905 for its peak position. For the next peak, amino-related component has gain 56.32068 for its peak position. For the fat peak, it has gain 51.09575 for its peak position that rank at the second place after the amino-related component peak. On the other hand, water peak has the peak position percentages of 44.48856, which rank at the fourth place after the carbohydrate peak, protein (amide I) and protein (amide I) peak, amino-related component peak and water peak.



**Figure 4.39:** Peak position for carbohydrates, protein (amide I), protein (amide II), fat and water in the chicken to rice ratio (2:1) sample.

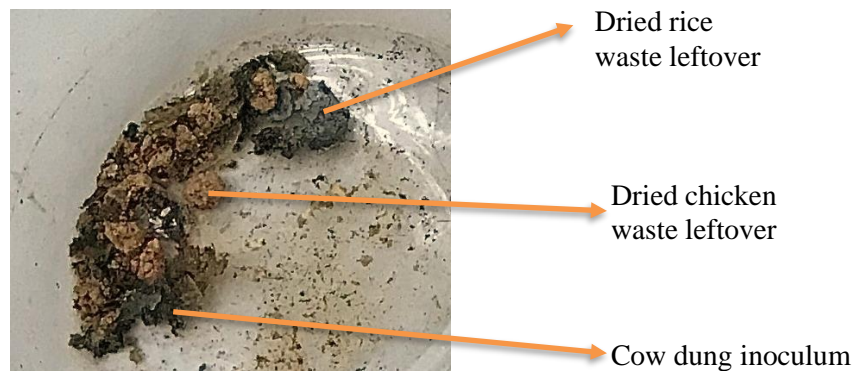
**Table 4.27:** Some information regarding the peak position and chemical component in chicken to rice ratio (2:1) sample

| PEAK POSITION | COMPONENT                              |
|---------------|--|
| 32.6288       | Carbohydrate                           |
| 46.00905      | Protein (Amide I) & Protein (Amide II) |
| 56.32068      | Amino-related component                |
| 51.09575      | Fat                                    |
| 44.48856      | Water                                  |

Figure 4.41 show the peak intensity for carbohydrates, protein (amide I), protein amide (II), amino-related component, fat component and water component in the chicken to rice ratio (2:1) sample. Peak intensity is considered as the area of concentration for that particular peak. As we can see in Figure 4.41, there are several red cross marks on certain peaks. This red cross mark indicated as the top value of the peak and the bottom value of the peak.

For the first peak that count from the left as shown in Figure 4.41, it is the carbohydrate peak and the top value for its peak position is 61.07565 while the bottom value for its peak position is 32.49058. Thus, the difference of these two peak position values have given the total peak intensity value of 28.58507. This indicates that there is high concentration of carbohydrate content is detected in this chicken to rice ratio (2:1) sample compared to others chemical content. Although the ratio of chicken is doubled to the ratio of rice, but the carbohydrates content that believed is came from rice is found higher compared to the protein content. After investigation, the high content of carbohydrates believed is come from the white flour that use to fried the chicken, which in this case, Kentucky Fried Chicken (KFC) was used as substrate. Before taking Kentucky Fried Chicken (KFC) into deep fried, basically on top of the chicken skin layer, there is covered with white flour. Pawan Kumar (2011) stated that there is 77.7% of carbohydrates composition from the white flour. Therefore. the high carbohydrates content of 28.5807 in the chicken to rice ratio (2:1) is make sense due to the addition of white flour in Kentucky Fried Chicken (KFC) as deep-fried purpose. And also, the carbohydrates content in rice leftover waste itself. In Figure 4.40, the brownish component is represented as the chicken leftover waste in dried condition.





**Figure 4.40:** Dried chicken waste leftover (brownish colour), rice waste leftover (greyish colour) and cow dung inoculum (charcoal blackish colour).

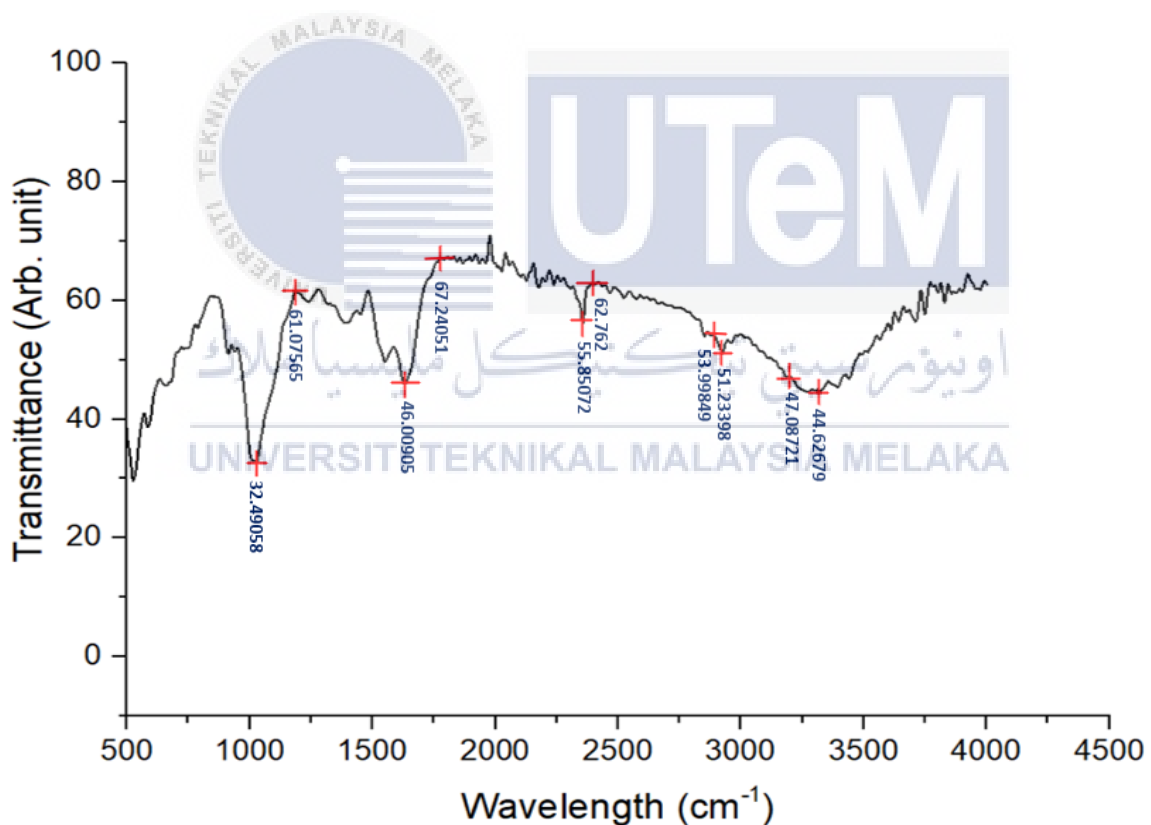
On the other hand, protein (amide I) and protein (amide II) component which have the total peak intensity value of 21.23146 is rank at the second place. This is due to the differences between the top peak position value of 67.24051 and bottom peak position value of 46.00905 as shown in Table 4.28. According to Hassan Mohammed (2020), there are approximately 20.02 of protein content in the chicken itself. Therefore, protein (amide I) and protein (amide II) are believed comes from the chicken leftover waste that have the ratio of two which in 369 grams. The amount of dried chicken leftover waste is appeared as brownish colour as shown in Figure 4.40.

For the third peak that count from the left, is the peak location for amino-related component. For this amino-related component, the top peak position value is 62.762 and bottom peak position value is 55.85072 as shown in Table 4.28. The differences between these two peak intensity values have given a total peak intensity value of 6.91128. This indicated that there are 6.91128 concentration of the amino-related component. The amino-related component is believed is the formation of amino acid that present in rice itself. According to Abdul Rohman (2014), rice contains essential amino acids for health promotion.

There is total peak intensity of 2.76091 of fat component in the chicken to rice ratio (2:1) sample too. This can be seen when there is a difference between the top peak position value of 53.99849 and bottom peak position value of 51.23398 as shown in Table 4.28. Fat component is believed that generate from the chicken skin itself, which in this case, the chicken skin from

Kentucky Fried Chicken (KFC) that have been go through deep fried process. H.Xin (2003) reported that chicken has 160 to 180g of skin on average, which is the part with the largest cholesterol and saturated fat contents. Not only that, if fried chicken with skin, it was found that is approximately 2227,18 arbitrary value of total fats would produce from it. From here, we know that fats that present in the skin and their possible effects on human health. Therefore, we should reduce the consumption of chicken skin especially of fried chicken skin.

For the fifth peak that count from the left as shown in Figure 4.41, it is the water peak and the top value for its peak position is 47.08721 while the bottom value for its peak position is 44.62679. Thus, the difference of these two peak position values have given the total peak intensity value of 2.46042. This indicates that there is relatively low water content is detected in this chicken to rice ratio (2:1) sample compared to others chemical content.



**Figure 4.41:** Peak intensity for carbohydrates, protein (amide I), amide (II) and fat component in the chicken to rice ratio (2:1) sample.

**Table 4.28:** Peak intensity information for carbohydrates, protein (amide I), amide (II) and fat component in the chicken to rice ratio (2:1) sample.

| PEAK INTENSITY    | TOTAL PEAK INTENSITY | COMPONENT                              |
|-------------------|----------------------|--|
| 61.07565-32.49058 | 28.58507             | Carbohydrate                           |
| 67.24051-46.00905 | 21.23146             | Protein (Amide I) & Protein (Amide II) |
| 62.762-55.85072   | 6.91128              | Amino-related component                |
| 53.99849-51.23398 | 2.76091              | Fat                                    |
| 47.08721-44.62679 | 2.46042              | Water                                  |

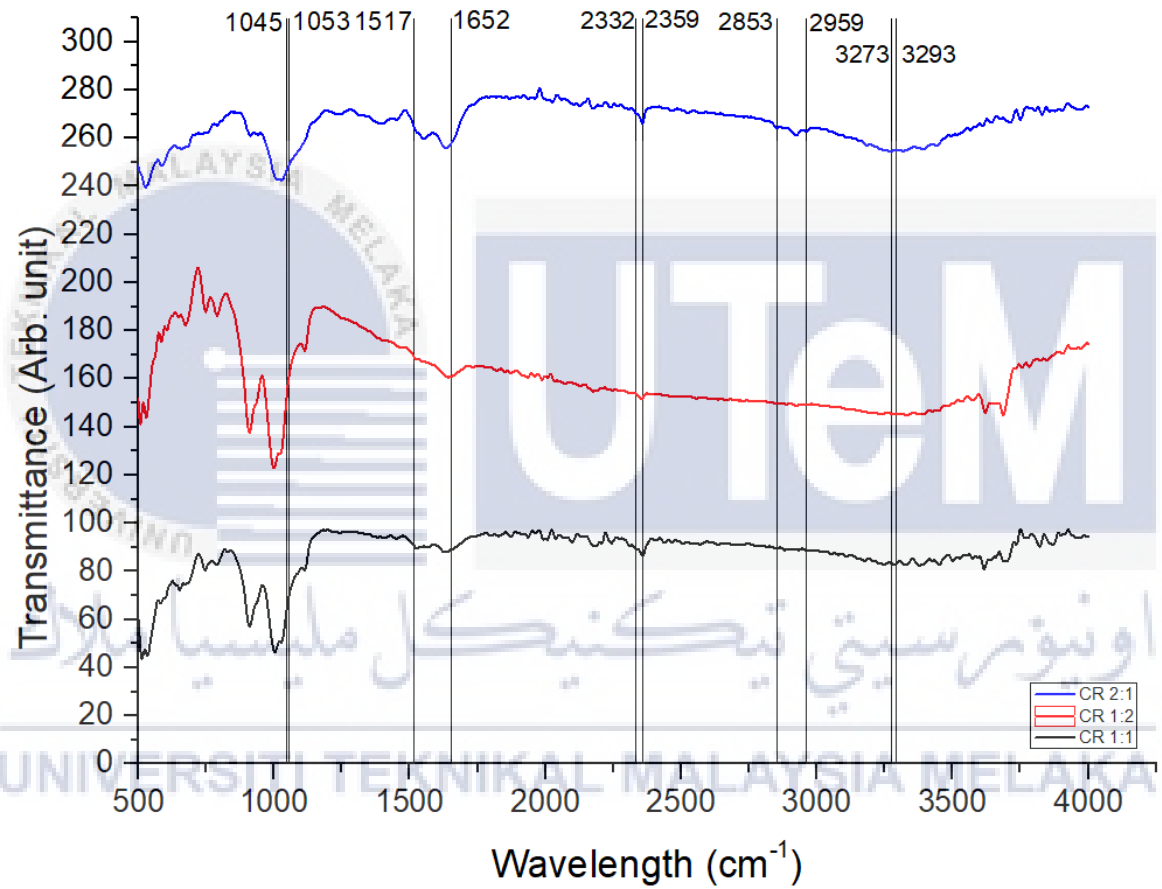
**iv. Comparison between Chicken to rice ratio (1:1), Chicken to rice ratio (1:2) and Chicken to rice ratio (2:1).**

The FTIR spectra shown in Figure 4.42 and the characteristics of functional groups in Table 4.29 illustrated the chemical differences the chicken to rice (1:1) sample, chicken to rice (1:2) sample and chicken to rice (2:1) sample. According to Huleihei (2002), the C-O stretching coupled with C-O bending of the C-OH of carbohydrates at  $1045-1053\text{ cm}^{-1}$  was assigned to chicken to rice (1:1) sample, chicken to rice (1:2) sample and chicken to rice (2:1) sample. The stronger relative intensities of these three peaks indicated chicken to rice ratio (1:2) is having higher carbohydrate content than chicken to rice (1:1) sample and chicken to rice (2:1). Apart from that, Schulz and Baranski (2007) stated that the absorption peaks at  $1517-1652\text{ cm}^{-1}$  resulted from stretching C=N, C=C, C=N guanine, C-C stretch of phenyl were associated with amide I and amide II of proteins. There are presence of protein (amide I) and protein (amide II) in all three samples as shown in Table 4.30.

From Table 4.30, we can see that the chicken to rice (2:1) sample is having the highest protein (amide I) and protein (amide II) content compared to the other two samples, which is 21.23146 of protein (amide I) and protein (amide II) content. Nandiyanto (2018b) reported that

the characteristic peaks at 2332-2359  $\text{cm}^{-1}$  were attributed to NH component caused by the amino-related component. There is presence of amino-related component in the chicken to rice (1:1) sample and the chicken to rice (2:1) sample. For the fat components in the samples, Yang (2005) stated that the absorption bands at 2853-2959  $\text{cm}^{-1}$  were ascribed to  $\text{CH}_2$  of lipids, asymmetric  $\text{CH}_2$  stretching mode of the methylene chains in membrane lipids. There are only one sample consist of fat component, which is the chicken to rice (2:1) sample. This might due to the presence of chicken skin that have gone through the deep-frying process. On the other hand, Doybeshko (2000) mentioned that there is presence of water content in the chicken to rice (1:2) sample and chicken to rice (2:1) sample at the wavelength of 3273-3293  $\text{cm}^{-1}$ . There is presence of stretching O-H symmetric chemical structure in the water content of the chicken to rice (1:2) sample and chicken to rice (2:1) sample.





**Figure 4.42:** Comparison between Chicken to rice ratio (1:1), Chicken to rice ratio (1:2) and Chicken to rice ratio (2:1).

**Table 4.29:** Basic information regarding to the chicken to rice ratio (1:1), chicken to rice ratio (1:2) and chicken to rice ratio (2:1).

| Wavelength (cm <sup>-1</sup> ) | Functional group   | Bnad Assignment                              | References   |
|--------------------------------|--|--|--|
| 1045-1053                      | C-O stretching coupled with C-O bending of the C-OH of carbohydrates   | Carbohydrate                                 | Huleihel, 2002   |
| 1517-1652                      | Stretching C=N, C=C, C=N<br>guanine<br>C-C stretch of phenyl   | Protein (Amide I)<br>&<br>Protein (Amide II) | Schulz and Baranski, 2007<br>Paluszkiewicz and Kwiatek, 2001 |
| 2332-2359                      | NH component   | Amino-related component                      | Nandiyanto, 2018b  |
| 2853-2959                      | CH <sub>2</sub> of lipids, Asymmetric CH <sub>2</sub> stretching mode of the methylene chains in membrane lipids | Fat  | Yang, 2005   |
| 3273-3293                      | Stretching O-H symmetric   | Water  | Dovbeshko, 2000  |

**Table 4.30:** Total peak intensity for the chemical components of each sample.

| Sample \ Total Peak intensity | Carbohydrate | Protein (Amide I) & Protein (Amide II) | Amino-related component | Fat     | Water   |
|-------------------------------|--------------|--|-------------------------|---------|---------|
| Chicken to rice ratio (1:1)   | 35.08552     | 5.60587                                | 6.32182                 | -       | -       |
| Chicken to rice ratio (1:2)   | 50.99774     | 7.69037                                | -                       | -       | 4.85549 |
| Chicken to rice ratio (2:1)   | 28.58507     | 21.23146                               | 6.91128                 | 2.76091 | 2.46042 |

## 4.5 Potential of Biohydrogen Production

This section was discussed the comparison study on biogas mini-reactor design for biohydrogen production. Then, the potential of biohydrogen production and future outlook was also elaborated in this section.

### 4.5.1 Comparison on mini reactor design for biohydrogen production

Random comparison study was made to evaluate the feasibility of our design. It can be summarized in Table 4.31. From comparison, our design was found capable to produce biohydrogen gases. Also, this result was validated by GC-TCD gas detection analysis. Referring to Table 4.31, with the substrate used chicken leftover waste and rice leftover waste, 10.84388% of biohydrogen gases is managed to be yield at the chicken to rice (1:2) sample. Also, chicken to rice (2:1) sample is capable to yield 3.17608% biohydrogen gases.

Table 4.31 shown most of the reactor design that have the stirred function which can give slurry a perfectly mixed. For instance, the impeller in continuous stirred tank reactor (CSTR) is utilised for effective mixing to get the desired result in the shortest period possible. According to Cahyari (2016), CSTR is able to yield 261 ml H<sub>2</sub>/g VS in the period of 32 days. This indicate CSTR would able to produce high amount of biohydrogen gases due to its stirred function. Hence, biogas reactor with the addition of stirred function will be considered as improvement for future study.

Since this study is aim to detected the gases present from the mini reactor design, thus quantification of gases will not be made. For future study recommendation, the present gases will be quantifying and the possibility to enhance biohydrogen generation will be further explore by varying related variable in this continuous study. In order to quantify the gases, present in the future, the reactor design can be adjusted to fulfil this situation. For instance, mini reactor can connect to the GC-TCD instrument to quantify the gases present in term of volume production. Other than that, the design of input for biogas reactor will be alter as it is found that the substrate is hard to be inserted into the biogas reactor.

**Table 4.31:** Comparison on reactor design and maximum biohydrogen production yield.

| No. | Reactor and Operating condition                           | Substrate  | Maximum Biohydrogen production yield      | References            |
|-----|---|--|---|-----------------------|
| 1   | <b>Our Design</b><br><b>(Chicken to rice ratio (1:2))</b> | Food waste<br>(Chicken leftover waste & Rice leftover waste) | 10.84388%                                 | This study            |
| 2   | <b>Our Design</b><br><b>(Chicken to rice ratio (2:1))</b> | Food waste<br>(Chicken leftover waste & Rice leftover waste) | 3.17608%                                  | This study            |
| 3   | Batch fermenter   | Cheese whey  | 6.35 ± 0.2 mol H <sub>2</sub> /mollactose | A. Mathur, 2016       |
| 4   | STR,<br>35± 1 °C<br>pH = 6.0 ± 6.9                        | Food waste   | 1.67 - 1.73 mol H <sub>2</sub> /molhexose | C. Moon, 2015         |
| 5   | CSTR,<br>70 °C<br>pH = 7.0 - 8.0                          | Anaerobic sludge   | 1.11 mol H <sub>2</sub> /molhexose        | Y. Zhang, 2013        |
| 6   | UASB,<br>37 °C<br>pH = 6.5                                | Glucose  | 1.44 ± 0.1 mol H <sub>2</sub> /molhexose  | B. Si, 2015           |
| 7   | ESBG,<br>35± 1 °C   | Activate d sludge  | 1.7 mol H <sub>2</sub> /molhexose         | X. Wang, 2009         |
| 8   | Batch, 60 °C  | Corn Stalk   | 89.3 mL H <sub>2</sub> / g dry biomass    | G. Kumar, 2015        |
| 9   | CSTR,<br>60 °C<br>pH = 5.5                                | Tofu processing waste  | 2.3 mol H <sub>2</sub> /mol glucose       | M.-S. Kim,2010        |
| 10  | TBSBR,<br>pH = 4.65–5.87                                  | Municipal wastes   | 1.67 mol H <sub>2</sub> /mol glucose      | R.G. Puhulwella, 2014 |
| 11  | CSTBR,<br>33.5 °C<br>pH = 5                               | Cow dung compost   | 2.15 mol H <sub>2</sub> /mol glucose      | X. Wu, 2010           |



|    |                                    |                          |   |                         |
|----|------------------------------------|--------------------------|---|-------------------------|
| 12 | Anaerobic CSTR,<br>37 °C<br>pH = 5 | Glucose                  | 1.3 mol<br>H <sub>2</sub> /mol<br>glucose         | D. Karadag, 2010        |
| 13 | ASBR,<br>37 °C<br>pH = 5.5         | Wastewater               | 2.89 ± 0.18 mol<br>H <sub>2</sub> /mol<br>glucose | S.R. Chaganti, 2013     |
| 14 | FBR,<br>40 °C                      | Municipal<br>sewage      | 4.26 ± 0.04 mol<br>H <sub>2</sub> /mol<br>glucose | C.-N. Lin, 2009         |
| 15 | Batch,<br>55 °C<br>pH = 7.0        | Activated sludge         | 1.25 mol<br>H <sub>2</sub> /mol<br>glucose        | B. Baghchehsaraee, 2010 |
| 16 | Batch,<br>37 °C<br>pH = 5.5        | Distillery<br>wastewater | 1000 ml<br>H <sub>2</sub> /L<br>medium            | E. Wicher, 2013         |
| 17 | CSTR,<br>37 °C<br>pH = 5.5         | Starch                   | 2.3 mol<br>H <sub>2</sub> /mol<br>hexose          | T. Doi, 2010            |

#### 4.5.2 Potential of biohydrogen production and future outlook

Based on the proposed mini-reactor design, it was discovered that the biohydrogen was detected in this study as shown in Table 4.32. As a result, our concept was determined to be viable in the end. However, owing to the ratio of one for chicken waste leftover and one for rice waste leftover, one of the examined samples did not detect hydrogen. It was possible that the content of chicken waste leftover and rice waste leftover in a 1:1 ratio with the fixed amount of cow dung inoculum is insufficient to produce hydrogen at this position ratio. Because the total input weight for the chicken to rice (1:1) sample is only 369 grams, the input weight for the ratio of one for chicken waste leftover and one for rice waste leftover is inadequate to generate hydrogen. In contrast, the total input weight of 553.5 grams for the chicken to rice (1:2) sample and the chicken to rice (2:1) sample could generate biohydrogen gases. As a consequence, since biohydrogen gases may be generated in the chicken to rice (1:1) sample, the total input weight for future study would be 553.5 grams rather than 369 grams.

According to Dong et al. (2009), food waste includes not just carbohydrates, but also proteins and lipids, among other nutrients. According to studies, carbohydrates are preferred for H<sub>2</sub> production in dark fermentation. Okamoto et al. (2000) found that rice, maize, and potatoes produced H<sub>2</sub> yields ranging from 19.3 to 96.0 mL/g volatile solids (VS), while protein yields from eggs, chicken, and meat-rich food waste were negligible. Based on the experimental findings, Lay et al. (2003) stated that the H<sub>2</sub> generation capacity of carbohydrate-rich solid waste was about 20 times more than that of fat- and protein-rich solid waste. This is because the rice waste leftover from the chicken to rice ratio (1:1) sample does not contain enough carbohydrates to start the reaction. In comparison, the ratio of two for rice waste leftover in the chicken to rice ratio (1:2) may produce 10.84388% of hydrogen gases as discovered by GC-TCD gas analysis. As a result, the ratio of one for chicken waste leftover and one for rice waste leftover were insufficient to produce hydrogen gases.

As demonstrated in Table 4.32, our design was capable of producing biohydrogen gases. However, within the scope of our research, we are just detecting the existence of hydrogen and not quantifying it into mol calculations. Perhaps as an enhancement, we will expand the research to include mol calculations. According to Table 4.32, the researcher will usually compute the biohydrogen based on the hydrogen per mol hexose (H<sub>2</sub>/mol hexose). The H<sub>2</sub> yield is expressed in three units: mol H<sub>2</sub>/mol hexose, mL H<sub>2</sub>/g volatile solids (VS), and mL H<sub>2</sub>/g chemical oxygen demand (COD). But due to this pandemic season, we are only able to detect the present gases in percentages with the help of GC-TCD instrument as shown in Table 4.32. Carbohydrates, on the other hand, seem to have a much greater potential for H<sub>2</sub> production than lipids and proteins. As a result, H<sub>2</sub> output on a hexose basis is a critical component in scientific assessment (Dong et al., 2009). The maximum H<sub>2</sub> production from hexose is 4 mol H<sub>2</sub>/mol hexose assuming all carbohydrates are converted into acetate. Acetate, however, cannot be the sole metabolite owing to thermodynamic limitations. As a result, in general, H<sub>2</sub> generation is less than 3 mol H<sub>2</sub>/mol hexose (Lalman et al., 2013).

The majority of the references make use of food waste as the primary substrate. Food waste (FW) accounts for 50% of total solid waste, according to Adema (2018). As a result, food waste has the potential to significantly harm the ecosystem. For example, consider water pollution, air pollution, and global warming. As a consequence, food waste

must be managed with care, with anaerobic digestion having the potential to reduce the amount of food waste transported to landfills. Aside from that, the conversion of food waste into hydrogen is one of the most promising methods. According to Hallenbeck PC (2002), hydrogen is produced through biological fermentation of organic substrates, which is then followed by a metabolic route. It is possible to employ light-dependent (photo-fermentation) or light-independent (dark fermentation) methods. The dark fermentation method is utilised in this instance. For your knowledge, anaerobic bacteria cultivated in the absence of light may generate hydrogen via dark fermentation (Arimi MM, 2015). Biohydrogen, which is produced via anaerobic fermentation, has great potential in terms of organic waste use since it minimises negative environmental effects while simultaneously offering renewable energy sources. The greatest amount of power generated by hydrogen, for example, is 237.2 kJ/mol, which may be produced by a fuel cell (Moreno-Andrade I, 2015). At the same time, it has no impact on the production of greenhouse gases (Zahedi S, 2013).

According to Table 4.32, several authors utilise cow dung and food waste as main substrates. Wu (2010) and Moon (2015) are the authors. Mixture of food waste (chicken waste leftover and rice waste leftover) and cow dung as inoculum was developed by integrating the research of Wu (2010) and Moon (2015). The existence of biohydrogen gases is shown by the addition of two wastes, as indicated in Table 4.32, namely food waste (chicken waste leftover and rice waste leftover) and cow dung. Due to time constraints and movement restriction orders in Malaysia, further research is unable to continue. So, for the time being, it is only able to describe our biogas reactor's ability to produce biohydrogen using GC-TCD gas detection. The research may be improved for future investigations by converting the percentage calculation of biohydrogen into mol calculations and quantifying the biohydrogen output. Also, fixed variable that referring to previous journal is used but our concern in this study is just to check the different in term of substrate ratio towards the potential of biohydrogen gases generation. All in all, there are only one variable to be tested in this study, which is different substrate ratio between chicken waste leftover and rice waste leftover. This is due to the time limitation in this pandemic season and the cost of gas testing is found to be very high. Also, from previous researcher study, researcher is found to be more interest to examine the effect of substrate in the biohydrogen gases production while the other parameter is being fixed as shown in Table 4.31. Apart from that, the inoculum in this study have been fixed due to the time limitation and movement

control order in Malaysia. Hence, the inoculum content may be varying instead of fixed for the next study.

**Table 4.32:** Percentage of hydrogen yield by each sample.

| Sample<br>(Chicken to Rice ratio) | Hydrogen yield<br>(%) |
|-----------------------------------|-----------------------|
| 1:1                               | -                     |
| 1:2                               | 10.84388              |
| 2:1                               | 3.17608               |

Fossil fuels remain the primary source of energy generation for the great majority of the world's population and have been used to create electricity for millennia. However, there are several drawbacks to using them. For instance, fossil fuels are non-renewable. Fossil fuels, which take millions of years to develop deep under the earth, do not appear to be replenished quickly enough for people to utilise indefinitely. As a result, when it comes to long-term energy sustainability, depending on fossil fuel reserves is not the greatest option. In 2012, fossil fuels accounted for 68% of all power generated globally. The total renewable energy generation, on the other hand, was 4862 TWh. And according to Behrouzi (2016), overall renewable energy is expected to reach 12851 TWh by 2021. The slow depletion of fossil fuel resources, costly recovery, and exponential rise in demand pose a major challenge to the sustainability of energy supply, it may be said here. Therefore, biohydrogen generation may be the key to reaching future sustainable renewable energy sources.

On the other hand, Nicoletti et al. (2015) examined the weighted percentages of hydrogen, carbon, methane, and octane in combustion flue gas as shown in Table 4.33. Apart from the production of nitrogen oxides, it is obvious from the data in Table 4.33 that the burning of hydrogen produces zero CO<sub>2</sub> and SO<sub>2</sub> emissions. The production of NO<sub>x</sub> is dependent on the temperature and duration of the flame (Geng, 2016). Because hydrogen has such a wide flammability range, its combustion may be impacted by how an engine is constructed, therefore the goal should be to decrease NO<sub>x</sub> emissions. Khan et al. (2016) have reported the Impacts on the environment that are additional to greenhouse gas

emissions. For example, anaerobic digestion process would produce biogas that contains methane. Apart from combustion, methane may be present in the liquid effluent, causing eutrophication, marine aquatic eco-toxicity, freshwater aquatic eco-toxicity, and other environmental issues. The creation of hydrogen from anaerobic digestion could address these severe environmental issues because hydrogen is not soluble in water.

The benefit of hydrogen as a clean energy source is that it minimises polluting emissions into the atmosphere. Thus, biohydrogen is clearly one of the finest alternatives to fossil fuel energy. Others that that, biohydrogen generation also has the added benefit of not endangering the environment during the process. Biohydrogen is thought to be one of the next-generation biofuels, with the potential to reduce fossil fuel reliance while also lowering greenhouse gas emissions from the energy and transportation sectors. Hydrogen's potential function as a clean fuel for fuel cells that produce near-zero emissions, as well as an intermediate energy carrier for storing and transporting renewable energy, is becoming more widely acknowledged.

**Table 4.33:** Pollution percentages in combustion flue gas for typical fuels.

| kg Pollutants /kg of fuel      |                 |                 |                 |                        |                  |
|--------------------------------|-----------------|-----------------|-----------------|------------------------|------------------|
| Fuel                           | CO <sub>2</sub> | SO <sub>2</sub> | NO <sub>x</sub> | Un-burns, particulates | H <sub>2</sub> O |
| H <sub>2</sub>                 | 0               | 0               | 0.016           | 0                      | 7                |
| C                              | 1.893           | 0.012           | 0.008           | 0.1                    | 0.633            |
| CH <sub>4</sub>                | 2.75            | 0.03            | 0.0075          | 0                      | 2.154            |
| C <sub>8</sub> H <sub>18</sub> | 3.09            | 0.010           | 0.0115          | 0.85                   | 1.254            |

## CHAPTER 5

### CONCLUSIONS AND RECOMMENDATIONS

#### 5.1 Conclusions

The first objective of this study was to characterize the specific food waste (chicken leftovers and rice leftovers) in terms of their composition using Fourier transform infrared (FTIR) analysis. FTIR analysis has successfully performed to analyse the composition of chicken waste leftover and rice waste leftover. Through this analysis, the nature of chemical composition for substrate used for biohydrogen production was fully understood. Hence, the first objective was successfully achieved. The second objective of this study was to evaluate the effects of substrate (chicken to rice waste leftover ratio) on the fixed content of inoculum during anaerobic digestion. The collected gases from chicken to rice ratios of (1:1), (1:2) and (2:1) were evaluated through GC-TCD analysis testing. Through gas detection testing, it was found that the substrate ratio of 1:2 promotes higher possibility of biohydrogen production. Thus, the second objective was also successfully achieved. The third objective of this study is to evaluate the potential of biohydrogen production using the proposed mini-reactor design. The biogas mini-reactor in this study was found workable and had potential as there was the presence of biohydrogen gases being successfully detected from the GC-TCD gas analysis test. Hence, the third objective was successfully achieved in this study. In overall, this study owned its significance value for green technology for bio-hydrogen production as alternative energy source from food waste resources, towards environmentally friendly circular economy for sustainable future.

## 5.2 Recommendations

There are several recommendations that could be suggested for further improvement of this research. Among all are as follows:

- a. Increase the retention time (HRT) for anaerobic digestion for possibility of methane production as green hydrocarbon source.
- b. Quantify the energy content of feedstocks using Bomb Calorimeter.
- c. Repeat the GC-TCD testing for six times for better accuracy and reliability.
- d. Evaluate the effect of varying inoculum content in biohydrogen production, to understand further the influence of microbes in promoting the bio-hydrogen production.

## 5.3 Sustainability Element

This study supports the green environmental concept because creating the potential commercial biohydrogen production by using waste resources as the main substrate. Also, by utilizing minimal cost, the biohydrogen mini-reactor design has been fabricated. By using cow dung as an inoculum, the reaction is promoted without using any chemical or synthetic reaction promoter or catalyst. Thus, our study is considered sustainable in terms of its approach, implementation and greater potential for green environment and sustainable waste to wealth circular economy.

## 5.4 Entrepreneur

In this research, the generation of biohydrogen utilising biogas mini-reactor shows cost savings when biohydrogen gases may be produced on a small scale. Furthermore, this mini biogas reactor is regarded as a portable piece of equipment with the lowest cost and maintenance while remaining competitive. Malaysians may use this study as a starting point for researching green and renewable energy options. This would provide Malaysians with an alternate source of income in the future. This is due to not depending exclusively on

hydrocarbons derived from fossil fuels, which would damage the environment and be expensive.

### **5.5 Life Long Learning**

Because this study is considered novel, there is still a lot of room for improvement that can be explored. As a life-long learning element, we need to further study the things that can improve our reactor design. For instance, substrate ratio, inoculum content, and so many other parameters can be studied.





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