DEVELOPMENT OF INTEGRATIVE FOOD WASTE TREATMENT AND LACTIC ACID PRODUCTION

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DEVELOPMENT OF INTEGRATIVE FOOD WASTE TREATMENT AND LACTIC ACID PRODUCTION

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ABSTRAK

Sisa makanan merupakan masalah utama yang merugikan ekonomi global sekitar 16,688 tan makanan dihasilkan setiap hari dalam ekonomi, cukup untuk memberi makan kepada 2.2 juta orang tiga kali sehari. Disebabkan oleh pelepasan gas rumah hijau yang dijana semasa pembuatan dan proses makanan, sisa makanan juga menyumbang kepada pemanasan global dan perubahan iklim. Sisa makanan ini mengandungi kepekatan tinggi karbohidrat, lipid, protein, sumber nitrogen dan vitamin. Sisa makanan telah dipilih sebagai substrat dalam kajian ini untuk mengekstrak menjadi asid laktik. Hari ini, populasi sisa makanan semakin meningkat dari hari ke hari dan ini menyumbang kepada masalah dalam beberapa bahagian dan tiada ketidakkonsistenan terhadap alam sekitar jika masalah ini dikawal dengan betul. Dalam kajian ini, botol 500 ml digunakan sebagai reaktor penapaian kelompok telah digunakan dan dua pecahan sisa makanan iaitu pecahan kanji (sisa roti putih) dan pecahan selulosa (sisa buah) dibandingkan pecahan sisa makanan yang boleh menghasilkan hasil yang lebih tinggi. Tambahan pula, tempoh penapaian, suhu dan pH awal untuk penapaian sisa makanan turut disahkan dalam kajian ini. Selain itu, peningkatan pengeluaran asid laktik dengan menambah budaya permulaan dalam sisa makanan juga diterokai. Semua pecahan sisa dan sisa makanan dikisar secara berasingan dalam air ternyahion suling untuk membentuk buburan sisa makanan (disterilkan dengan autoklaf), pH awal diselaraskan kepada pH 7 dan ditapai dibiarkan pada suhu bilik. Sisa makanan yang paling berkesan berdasarkan hasil asid laktik ialah sisa roti putih. Masa penapaian yang paling sesuai ialah 15 hari dan suhu 36.4^{o}C. Keputusan Permintaan Oksigen Kimia (COD) dan Permintaan Oksigen Biokimia (BOD) menunjukkan kecekapan rawatan sisa makanan manakala bacaan pH menunjukkan kehadiran asid dalam sampel. Keputusan menunjukkan bahawa kedua-dua pecahan sisa makanan mempunyai kehadiran asid laktik tetapi dalam kepekatan yang berbeza.

ABSTRACT

Food waste is a major problem that costs the global economy around 16,688 tons of food are produced every day in the economy, enough to feed 2.2 million people three times a day. Due to greenhouse gas emissions generated during food manufacturing and process, food waste also contributes to global warming and climate change. This food waste contains a high concentration of carbohydrates, lipids, proteins, nitrogen sources and vitamins. Food waste have been chosen as substrate in this study to extract become lactic acid. These days, food waste population becomes increasing day by day and this donate to problems in several part and no inconsistency into environment if this problem bot controlled correctly. In this study, 500 ml bottle act as the batch fermentation reactor was used and two fraction of food waste namely starchy fraction (white bread waste) and cellulosic fraction (fruit waste) was compared which food waste fraction could produce the higher yield. Furthermore, the duration of fermentation, temperature and initial pH for food waste fermentation were also confirmed in this study. Moreover, improvement of lactic acid production by adding a starter culture in food waste was also explored. All waste fractions and food waste were grinded separately in distilled deionized water to form food waste slurry (sterilized by autoclaving), initial pH adjusted to pH 7 and fermented was left at room temperature. The most efficient food waste based on yield of lactic acid was white bread waste. The most suitable fermentation time is 15 days and temperature are 36.4^{o}C. The result of the Chemical Oxygen Demand (COD) and Biochemical Oxygen Demand (BOD) indicate the efficiency of the food waste treatment while the reading of the pH indicate the presence of the acid in the sample. The result indicated that both fraction of food waste has the presence of the lactic acid but in different concentration.

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LIST OF SYMBOLS

%	Percent
^{o}C	Degree Celcius
kPa	Kilopascal
N	Normality
M	Mol
ml	Millilitre
min	Minute

LIST OF ABBREVIATIONS

FAO	Food and Agriculture Organisation
LAB	Lactic Acid Bacteria
MSW	Municipal Solid Waste
PLA	Poly-Lactic Acid
BOD	Biochemical Oxygen Demand
COD	Chemical Oxygen Demand
BW	White Bread Waste
FW	Fruit Waste

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CHAPTER 1

INTRODUCTION

1.1 Project Background

One of major problem encountering today is the discharge of large amount of food waste in the environment. Malaysia's Petronas Twin Towers standing about 451.9 meters tall from the ground. Now just to imagine 16 of these twin towers being filled with the thousand kilo tons of food waste. Obviously, this situation could become a reality if we do nothing to reduce or solve the problem of food waste. According to the Solid Waste Management and Public Cleansing Corporation (SWCorp) by 2020, the total amount of food waste produced will be higher to fill up 16 of the twin towers. According to (Yui Fai Tsang, 2019), from United Nations Food and Agriculture Organization (FAO), approaching to 1.3 billion tons of food waste result in severe health problem and cost. To support this statement, eating spoiled food will be result in severe health problem also cost increase. Moreover, food waste contains a high concentration of carbohydrates, lipids, proteins, nitrogen and vitamins.

Conforming to this study, the way to minimize and reduce the amount of food waste, including using food waste to produce lactic acid and produce or generate electricity. Integrative techniques have proven for maximization of energy recovery from food waste including this technique which is electro – fermentative in form of bio – electricity with simultaneous production of value – added chemicals which is lactic acid.

Furthermore (Fabian Bonk, 2017) said, over the last few years, alternative technologies such as the carboxylate platform have been developed to produce value – added products from food waste. Others, to produce the carboxylic acid is via undefined mixed culture of bacteria in the carboxylate platform (Fabian Bonk J. R., 2015).

In this study, development of integrative food waste treatment and lactic acid production which is applying the fermentation process to extract lactic acid from food waste for effectiveness of microbes in this research. The diverse applications of lactic acid, combined with the growing demand for environmentally products (Tsz Him Kwan, 2017). Lactic acid is industrial important platform chemical which is used a pH regulator and preservative in the food and beverage industries, cosmetics, textiles, pharmaceutical and chemical industries. Moreover, function of lactic acid to produced commercially by fermentation of carbohydrates such as glucose, sucrose or lactose and chemical synthesis.

1.2 Problem Statement

Food waste is always being a concern and is the major problem around the world. The increasing of food waste disposal population and development into environment have significantly risen the fresh air requirement. However, this easily leads to the air pollution that is caused by human, industrial, food factory, restaurants and domestic waste trickle into fresh air bodies. Since fresh air bodies are polluted because of uncontrolled food waste disposal, it becomes hardly to be reduced. Food waste has a high energy potential, biodegradability and significant energy recovery. However, due to the inconsistency of the composition on daily basis, food wastes may cause uncontrolled surrender and generation.

According to (Tsz Him Kwan, 2017), approximately 300,000 to 400,000 metric tons (MT) per year of food waste with industrial application grow at a rate of 18.6 percent per year. This result is the polluted fresh air will consequently affect human health, risk into respiratory system, heart disease and the environment. Furthermore, human require a safe place environment and basic sanitation from fresh air. People need a good environment and clean fresh air and sanitation to maintain their health. As a solution, the efficient utilization of food waste resources recommended, it is replacement ways to landfill which is food waste can be composed and used to produce energy or fuel.

Microbial fuel cell (MFC) is waste management tool which anaerobic bacteria are used to produce energy/electricity from waste. (Daniel Pleissner, 2017) classified that, the sources of food waste depend on meat, noodles, potatoes, vegetables, fruits, bread and cake. His statement goes to show that food waste is everywhere and around us. People must be more responsible in order to help decrease the food waste disposal. In this study, microbial fuel cell (MFC) with lactic acid and food waste treatment interact to transform waste become energy saving.

Microbial fuel cell (MFC) is providing more a low-cost alternative to conventional. The purpose of this study, to integrate the new technique proven for recover energy from food waste source and extract lactic acid production from waste. Moreover, microbial fuel cell used because it is the valuable waste management tools recently due to the rise in the demand of bioelectric energy and environment problem that MFC is the new alternative energy sources to minimize/reduce energy compared to traditional activated sludge process can effectively remove organic pollutant.

Microbial fuel cell is recently novelty technology to full fill mission because can directly convert biodegradable materials into renewable energy (Tyler Huggins, 2013). However, using the conventional method which is anaerobic digestion contribute the disadvantages when not able and fail to function low concentration substrate and not able to focus on the reactor configuration, power density and the materials cost. Therefore, food waste is a rich substrate for the production of lactic acid and the fact that the production of lactic acid via unsterilized food waste with additional nutrient and continuous pH adjustment. In Global, lactic acid demand was estimated become 1,947.2 kilo tons in 2018 and it expected to increase by 16.2% per year started from 2019 to 2025 due to increased sales of medicines and perfumes (Mohamed Ali Abdel Rahman, 2019).

This electro – fermentative lactic acid is more effective use of waste organic matter compared to conventional method because anaerobic degradation does not allow use of the whole prospects of food waste as functionalized realization of material use of food waste. The lactic acid fermentation from food waste offers the potential for practical application but are still on early – stage. Therefore, much more efforts are required to used food waste producer to yield of lactic acid. Nonetheless, the higher efficiency of substrates and their satisfactory stability for the production of lactic acid has yet to be investigated.

1.3 Objective of Study

This project has objectives as below:

i. To perform a validation test on the reactor using nutrient medium & food waste for lactic acid.

ii. To optimize the lactic acid production and food treatment efficiency

1.4 Scopes of Study

This study has been conducted in cafeteria in University Malaysia Pahang, Gambang Campus that are at Kolej Kediaman 3. The food waste collected from the cafeteria and have been store before weighed according to composition that has been made. Food waste that has collected will be process using blander and fill in the fermentation bottle. After that, will analyse the parameter that required before fermentation process and keep the process in 15 days. In order to produce lactic acid fermentative is designing of integrated electro fermenter for lactic acid. Besides, the validation test will be conducted on the reactor using the food waste. In addition, detecting and recording the temperature which is to produce maximum concentration and maximum yield. Moreover, measuring and calculating the parameter which is BOD, COD, pH, Jar test and High-Performance Liquid Chromatography (HPLC) for observe lactic acid. Last but not least, microbial fuel cell will generate after putting the balance of substrate after the fermentation process to know that food waste can generate the electricity or not.

1.5 Importance of Study

By identifying the type of composition of food waste that produced in study area, so the solution has been made on how to reduce and managed food waste. The type of waste and the amount generated will be studied so that the existing system can be improved in order to reduce the solid waste in this study. Reuse, reduce and recycle (3R) campaigns can be improved and expanded so that awareness arises in everyone because this is the best way to reduce waste. The decreasing of food waste such as vegetable, bread and rice and so on can reduce the air and smell pollution into the environment also in the cafeteria area as well as will be clean and nurture importance of green technology with each individual.

CHAPTER 2

LITERATURE REVIEW

In this subsection, subjects such as food waste, lactic acid, method of production, related research that are introduced in Chapter 1 are advance exhausted to cover definition, history, issues, detailed method and recorded inquire about related to this consider.

2.1 Food Waste Generation

Food waste is a major problem that costs the global economy around 16,688 tons of food are produced every day in the economy, enough to feed 2.2 million people three times a day. Furthermore, this food waste contains a high concentration of carbohydrates, lipids, proteins, nitrogen sources and vitamins. Food waste have been chosen as substrate in this study to extract become lactic acid. These days, food waste population becomes increasing day by day and this donate to problems in several part and no inconsistency into environment if this problem bot controlled correctly. Furthermore, food waste may contain few sources depending on variety types which is meat, noodles, vegetables, rice, fruits, bread and also potatoes (Daniel Pleissner, 2017). Due to greenhouse gas emissions generated during food manufacturing and process, food waste also contributes to global warming and climate change. According to the United Nations Food and Agriculture Organization (FAO), approximately 1.3 billion tons of food are lost or wasted globally each year (Yui Fai Tsang, 2019). Furthermore, consuming spoiled food will result in severe health problems and costs. Figure 2.1 shows that the annual average of food waste production.

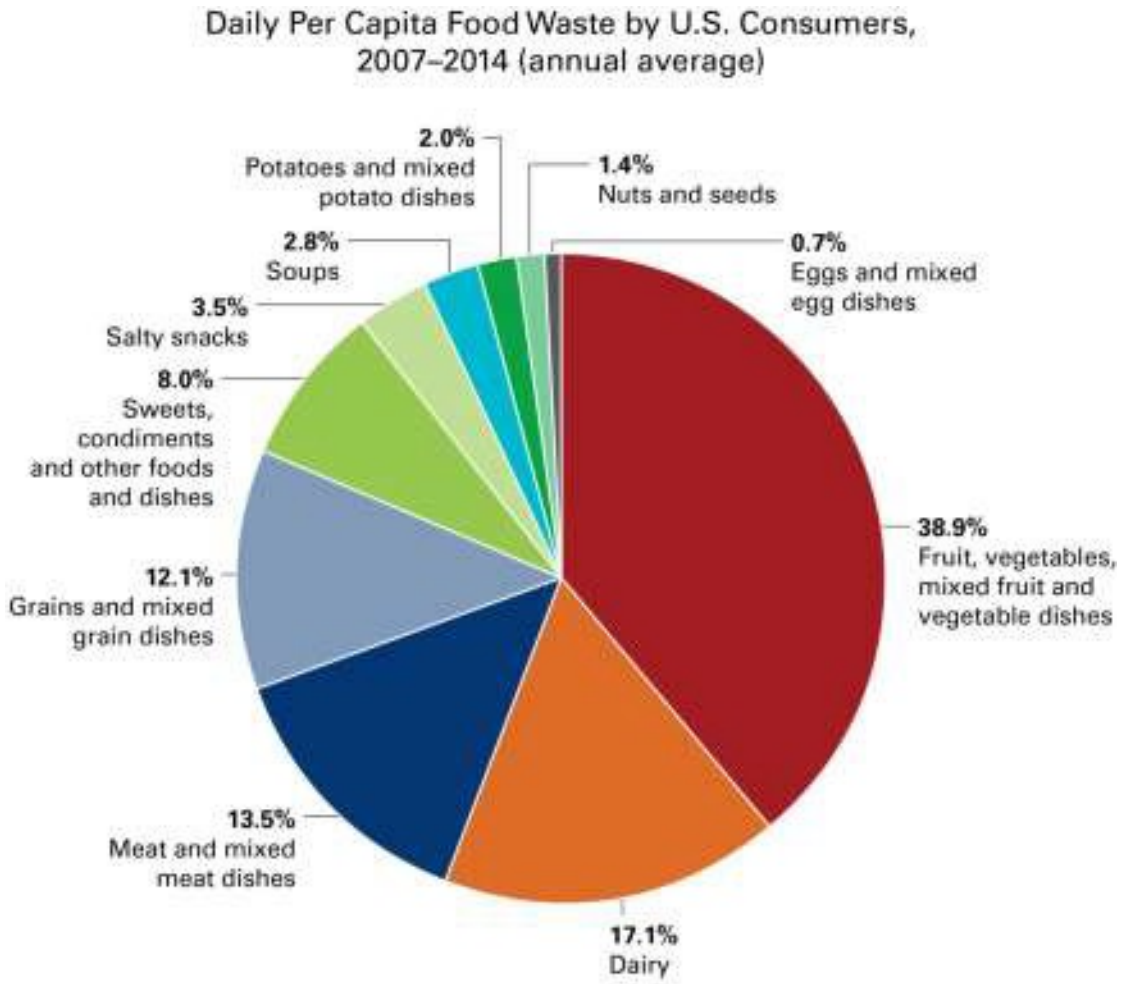

Figure 2.1 Annual average of food waste production

2.2 Municipal Solid Waste in Malaysia

Malaysians discarded up to 33,000 metric tonnes of waste per day last year, costing the government approximately RM1.2 billion in waste collection (Bernama, 2014). Currently, the country is reliant on landfills, with nearly 85 percent of waste collected ending up there. Malaysians are expected to generate approximately 49,670 metric tonnes of waste per day by 2020 (Yong, 2019). There are now 170 waste disposal sites in Malaysia, although only 14 of them are designated as sanitary landfills. Due of its voluminous nature, MSW removal and treatment are a major issue in many nations. The traditional method of Municipal Solid Waste (MSW) management is landfill disposal, although industrialised nations are rapidly reducing their reliance on it owing to environmental concerns (Siti Salwa Khamis, 2019). Malaysia is increasingly running out of suitable land for development, despite the fact that it is not as obvious as it appears (Kadir S. A. S. A., 2013).

Rapid economic growth in Asia's developing countries has resulted in serious waste disposal issues as a result of uncontrolled and unmonitored urbanisation (Waste Management, 'Industry as a partner for sustainable development, 2002). The absence of financial and human resources educated in waste management procedures in the areas of collection, transportation, processing, and final disposal exacerbates the situation. In most situations, components like solid waste recovery, reuse, and recycling are excessively demand and supply driven or chaotic (Shapkota et al, 2006). Despite increased recycling efforts, only 17.5 percent of Malaysians recycle their waste (2017). The problem is exacerbated by the fact that the waste segregation programme is still in poor shape and will take some time for citizens to fully implement. Many countries, particularly developing countries, continue to manage their waste in landfills.

Some wealthy countries use incineration to eliminate their waste and the energy that is created as a result of the process. In Malaysia, landfilling accounts for nearly 85% of MSW management, while incinerators are only used on a small scale (Kadir S. A. S. A., 2013).

Furthermore, existing incinerator technology is deemed ineffective for handling domestic MSW due to cost, the waste contains high moisture content, and mixed waste is sent to the incinerator without separation. Not only does incinerating waste produce little extra value, but it also pollutes the environment. There is a growing demand for competitive and renewable alternatives to primary resources in manufacturing and fossil fuels in energy generation. At the same time, many countries, particularly developing ones, continue to landfill the majority of their MSW, posing serious environmental risks and wasting a precious resource. A municipality can reduce waste's environmental impact while also increasing revenue from recycling and energy sales by converting MSW into a value-added product (Siti Salwa Khamis, 2019).

2.3 Fermentation of Lactic Acid

Lactic acid is one important product of interest due to its utilization in the manufacture of food/beverages, medicines, cosmetics, leather and textiles (John et. Al, 2019). Traditionally, lactic acid is produced through the fermentation of carbohydrates such as glucose, starch and molasses. Low - cost lactic acid is required for the successful commercialization of cost-effective lactate esters as alternatives to those derived from petrochemical sources. The production of low – cost lactic acid necessitates the use of inexpensive and easily accessible substrates

Since lactic acid or 2-hydroxypropionic acid can be synthesized chemically or biologically, non – toxic compound that has been widely used in the food, pharmaceutical and chemical industries (Roberto Mazzoli, 2014). Lactic acid isomer is preferred on food products because of the presence of dehydrogenase lactate in humans where lactic acid isomer is sometimes dangerous to human metabolism and can cause acidosis and decalcification (Emmanuel Alepu Odey, 2018). (Jialing Tang, 2016) concluded that lactic acid can produce by fermentation under sterile condition using various Lactobacillus strains such as L. manihotivorans, L. paracasei and L. plantarum. More recently, the production of lactic acid through microorganisms has been well develop in recent years (Emmanuel Alepu Odey, 2018). Fermentation under sterile conditions using Lactobacillus strains where function lactobacillus is to break down food and absorb nutrient. These can be in the form of refined (ex. Glucose, sucrose and starch) or complex (cellulose, whole cereal grains and waste materials). However, it often uses fermentation processes involving relatively expensive substrates, such as yeast extract, glucose or lactose.

Lactic acid L isomer is preferred in food products because the presence of dehydrogenase lactate in humans, where's lactic acid D isomer is sometimes dangerous to human metabolism and can cause acidosis and decalcification (C.M Narayanan, 2017). Furthermore, developing competitive bioprocesses to meet the current demand of lactic acid, there is a need to utilize the inexpensive substrate and nitrogen supplements to reduce the production cost (Raymond Redcorn, 2016). (Emmanuel Alepu Odey Z. L., 2018) have said, total elimination of fecal microorganisms in sludge by fermented food waste takes a long time (7 – 14 weeks) compared with hydrated lime (about 1h) and urea treatment (about 4 days). The energy source produced by starch hydrolysis to be specific glucose, is required by most microorganisms during fermentation in arrange to make the chemicals that are value-added items. One of the chemicals of interest produced via fermentation of lactic acid, basically created by lactic acid bacteria.

2.4 Importance of Lactic Acid

Lactic acid has been portioned of our food system for thousands of years, however the instrument for conservation and sensory qualities offered by these bacteria was not understood until 19th century with mechanical propels in microbiology. In the food industry, lactic acid is widely used in almost all branches of the food industry to regulate pH, provide aroma and improve the flavour and microbial quality of foods (Emmanuel Alepu Odey B. O., 2018). It is alight acidity makes it a good acidulant in salads, pastries and drinks. In the industry, lactic acid is used as an electrolyte in many parenteral medications (Emmanuel Alepu Odey B. O., 2018). Furthermore, the versatile applications of poly lactic acid (PLA) for which optically pure (L+) lactic acid acts as a precursor have propelled its current market expansion (Avinash Thakur, 2018).

Lactic acid is the most widely used in acid in several industries such as the pharmaceutical, food and chemical industries (Mostafa Ghasemi, 2016). It is estimated that global demand will be 130,000 to 150,000 (metric) tons per year (Mostafa Ghasemi W. R., 2016). Environment friendly products leas to a global production currently produce 300,000 – 400,000 metric ton (MT) per year and a growing industrial application at a rate of 18.6% per year (Kactic acid market application (biodegradable polymer, food & beverage, personal care & pharmaceutical) & polylactic acid market by application (packaging, agriculture, automobile, electronics, textile) & by geography - global trends & forecasts to 20, 2015). Lactic acid is one of the most widely used chemicals, not only in food but also in the medical, pharmaceutical, plastics and cosmetic industries.

2.5 Uses of Lactic Acid

Lactic acid, 2 – hydroxyl propanoic acid, is widely important product of interest due to its use in the production of foods/beverages, medicines, cosmetics, leather and textiles (Salma Aathika Abdur Rawoof, 2021). Figure 1 shows the lactic acid demand in various sectors based on the global lactic acid market revenue in 2018. Despite this, lactic acid is used in the food/beverage industry for its acid property in the production of soft drinks, candles, bakery items, milk productions, jams and jellies. It is also used in the beauty industry as a moisturizer due to its ability to retain water because of its ability to inhibit the development of tyrosinase, it is used as a skin lightener and rejuvenator. Lactic acid is also used in the medical field to make topical ointments, lotions, parenteral solutions, surgical sutures and prostheses.

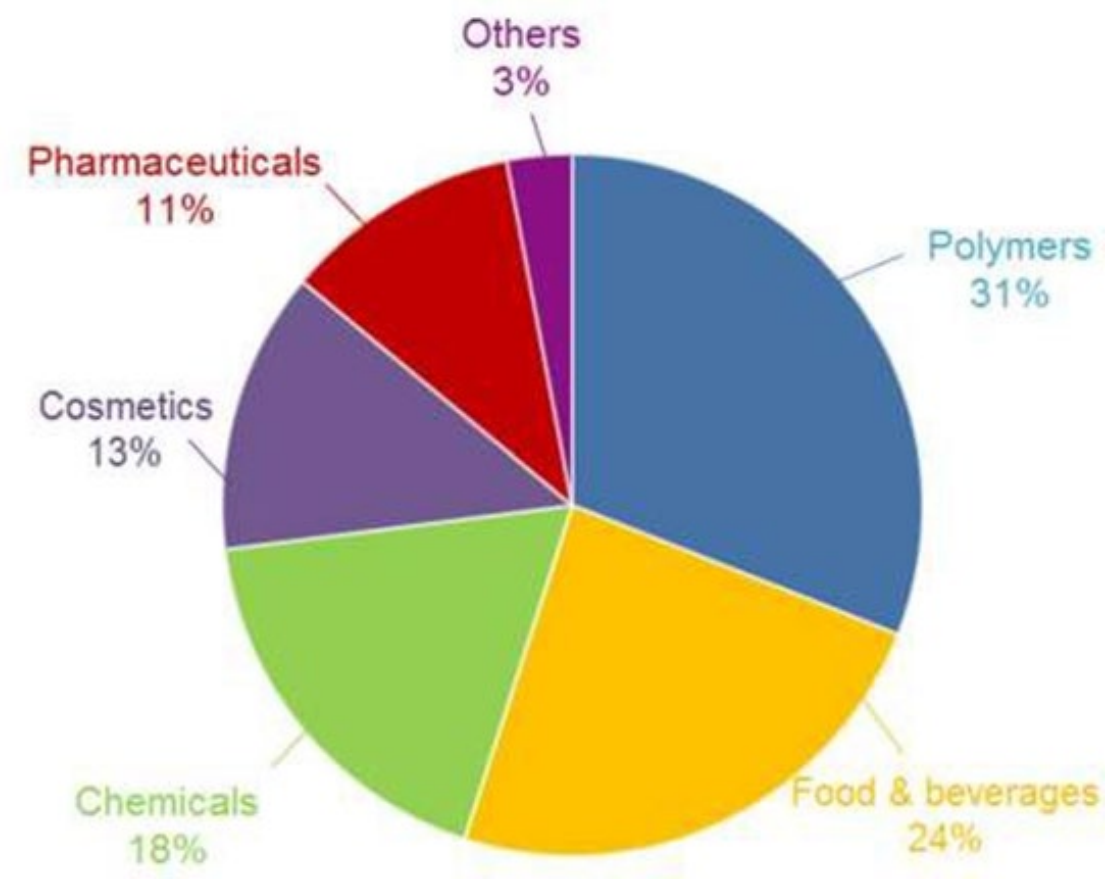

Figure 2.2 Demand for lactic acid (Global lactic acid market revenue in 2018)

Lactic acid (CH_3-CHOHCOOH), also known as 2 hydroxy propionic acid is a chemical compound that exists in two enantiomeric forms L (+) lactic acid and D (-) lactic acid, each with their own application. Furthermore, polymerization of lactic acid results in the formation of dilactide as an intermediate, resulting in poly lactic acid (PLA). Poly lactic acid is a product of lactic acid polymerization which can be accomplished through two methods, chemical and fermentation. This polymer, which is commercially used as a bioplastic, can be used in a variety of applications ranging from clothing fibres to films to paper coatings. Besides, lactic acid, a mixture of two isomers will be produced as a result of the chemical synthesis process (Yanty maryanty, 2021). To produce lactonitrile, an acetaldehyde reaction with hydrogen cyanide in the presence of a catalyst is used. The reaction takes place in the liquid phase at high atmospheric temperatures. Figure 2 shows a schematic illustration of lignocellulos produce lactic acid.

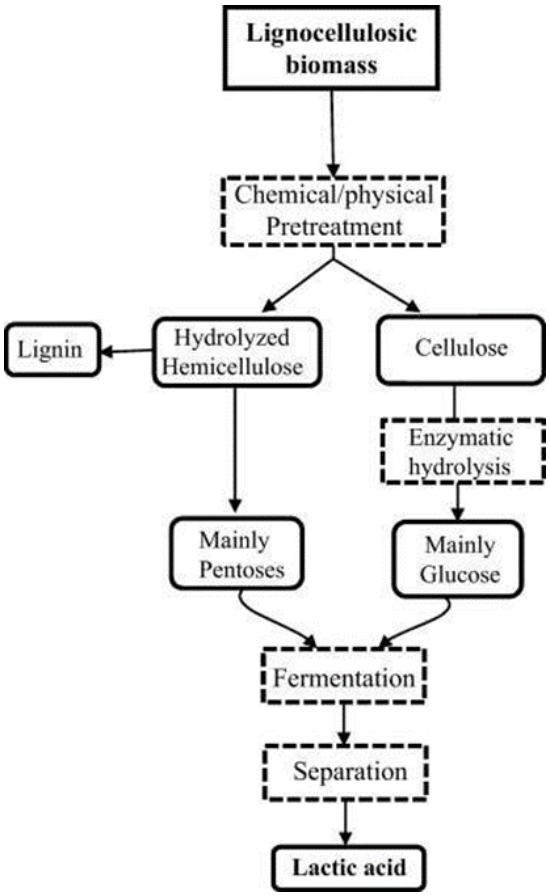

Figure 2.3 Schematic Lignocellulos produce Lactic Acid.

Fermentation method is an energy-producing process that is characteristic of anaerobic bacteria. The fermentation process will result in the production of specific lactic acid, such as L (+) lactic acid or D (-) lactic acid (Yanty maryanty, 2021). Figure 3 is the flowchart depicting the steps involved in the fermentation-based production and purification of lactic acid. Lactic acid is produced by bacteria by utilizing simple sugars such as glucose, lactose, and galactose without the use of a heating process. Nowadays, microbial conversion-based compound production has emerged as an important research area. Optically pure LA (D-LA or L-LA) can be produced using a fermentation method based on specific LA bacteria (LAB), resulting in a reduction in the time and cost of the recycling process (Guravatar Singh Mann, 2019). Fermentation processes are classified into three types, batch fermentation, fed batch fermentation and continuous fermentation (Sundus Riaz N. F., 2018). Batch and fed batch fermentation yield high lactic acid concentrations, where continuous fermentation yields higher productivity. Fermentation is typically performed at a controlled temperature and pH level (Sundus Riaz N. F., 2018). Both bacteria and fungi can produce lactic acid through fermentation, but the yield of lactic acid produced by fungi is significantly lower than the yield of lactic acid produced by bacteria (Sundus Riaz o. F., 2018).

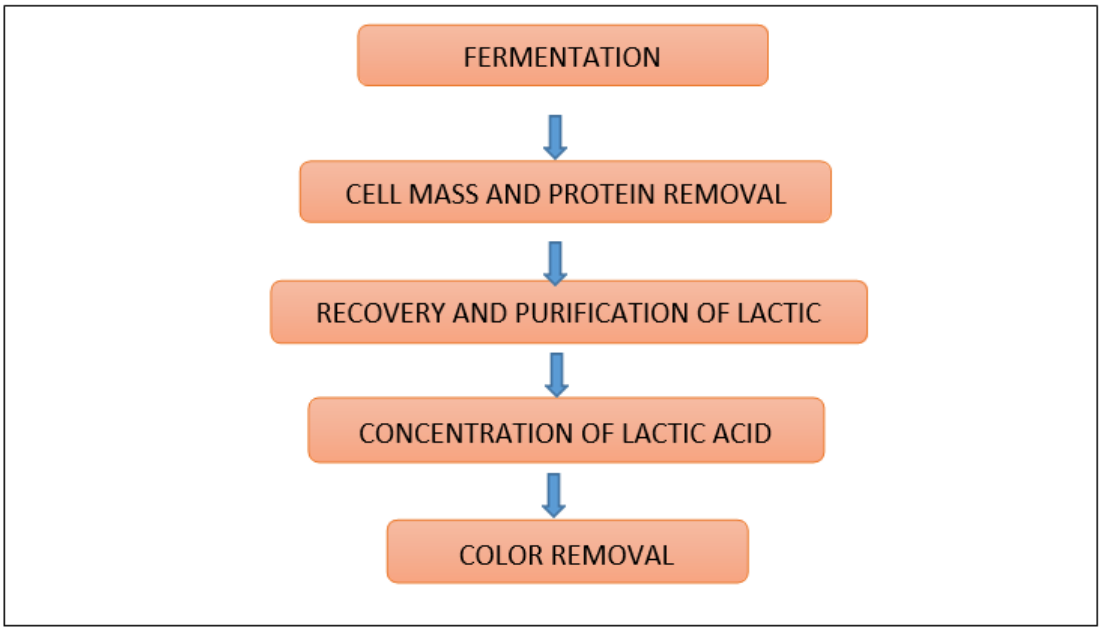

Figure 2.4 Flowchart of fermentation production and purification of lactic acid

2.6 Lactic Acid Producing Microorganism

The physical properties and characteristic of PLA products are determined by the type of lactic acid isomer used, which is determined by the organism used to produce it. The fermentation pattern as well as the isomer produced by selected lactic acid producing bacteria and fungi is the microorganisms and their yield of lactic acid production by fermentation. Homo fermentative LAB produce lactic acid in the absence of other metabolic by products and have a theoretical conversion of 1 mole glucose to 2 moles. Hetero fermentative LAB produces lactic acid as well as other by product such as ethanol, diacetyl and carbon dioxide. Thus, conversion of 1 mol glucose results in less than 2 mol lactic acid.

2.6.1 Types of Microorganism

Bacteria and fungi are the two types of microorganisms used in fermentation. The type of microorganisms to use is determined primarily by the carbohydrates to be fermented as microorganism's metabolism differs with various sources (Andrea Komesu, 2017). Lactic acid bacteria (LAB) are common microorganisms that can be found in any environment that is high in carbohydrates such as plants, fermented foods and the mucosal surfaces of humans, terrestrial and marine animals (Pnangionta Florou -Paneri, 2013). Lactic acid is produced by glycolysis pathway under anaerobic conditions and this compound can be produced from hexoses and pentose lactic acid bacteria (LAB) metabolism pathways (Elahe Abedi, 2020). This compound can be synthesized via the hexoses and pentose lactic acid bacteria (LAB) metabolism pathways as shown in **Figure 2.5**.

The yield and productivity of lactic acid are affected by pH (3.5 – 9.6), temperature (5–45 °C). These microorganisms are used to break down food and absorb nutrients. Lactobacillus strains are commercially important among lactic acid bacteria (LAB) strains due to their high acid tolerance, yield and productivity and can be engineered for the selective production of L/D lactic acid (Elahe Abedi, 2020). Exiguobacterium, for example is a bacilli genus with the alkaliphile Exiguobacterium sp. Strain 8-11-1 producing optically pure l-lactate in nonsterile fed-batch fermentation with a productivity of 8.15g/L/h under 80 g/L glucose concentration and using NaOH as a neutralizing agent (Elahe Abedi, 2020).

There are many different types of LAB microorganisms, which are the presence of nutrients such as amino acids, peptides, nucleotides and vitamins and the lactic acid bacteria (LAB) strains from the genus have been use, so for strains from the genus Leuconostoc, Lactococcus, Lactobacillus, Streptococcus and Carnobacterium (Elahe Abedi, 2020).

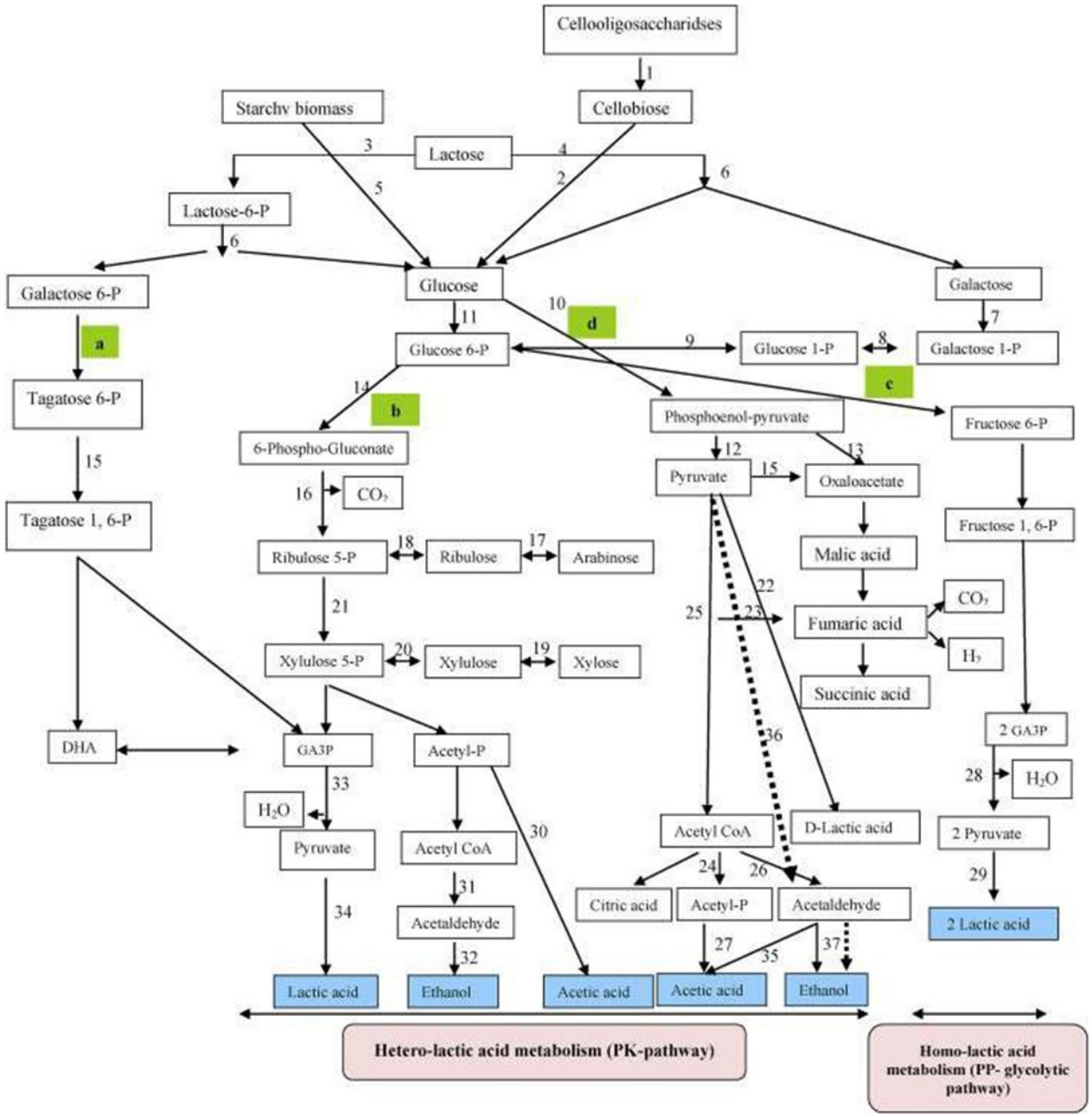

Figure 2.5 Lactic acid bacteria (LAB) metabolism pathways

CHAPTER 3

METHODOLOGY

3.1 Introduction

This section explains the research framework. Materials, experimental setup, inoculum, analytical methods have been demonstrated clearly. The details of the experimental outcome have been discussed further in the relevant chapters. Figure 3.1 elaborates the research framework to provide a crystal-clear view on the experimental strategy.

3.2 Batch Fermentation

Batch fermentation is a process where all the substrate and nutrients are mixed into the vessel after the addition of inoculum or inoculation occur with specific controlled environment until maximum concentration of the product desired are achieved. Lactic acid production can be accomplished by batch fermentation using microbes and repeated-batch fermentation using immobilized cells could offer several advantages such as higher fermentation rate, protective effect against possible inhibitors, decreased inoculum preparation processing cost, and reduced product contamination. (Chien-Chung Chen, 2019)

3.2.1 Food Waste Collection

Food waste (starch contained preferably) was collected from cafe around University Malaysia Pahang Campus and then blended by using a kitchen blender right after the collection. The food waste was then stored in laboratory refrigerator with temperature below -4^{o}c until the used in the experiment (Glass Door Chiller Chill-1050, Tech-Lab Scientific Sdn. Bhd, Malaysia) to prevent microbial activity such as biomass degradation occur before the actual test run to purify the waste from the presence of the impurities (their close association with lignin and lack of production of a hydrolytic enzyme by lactic acid producing strains.

3.3 Flowchart Methods

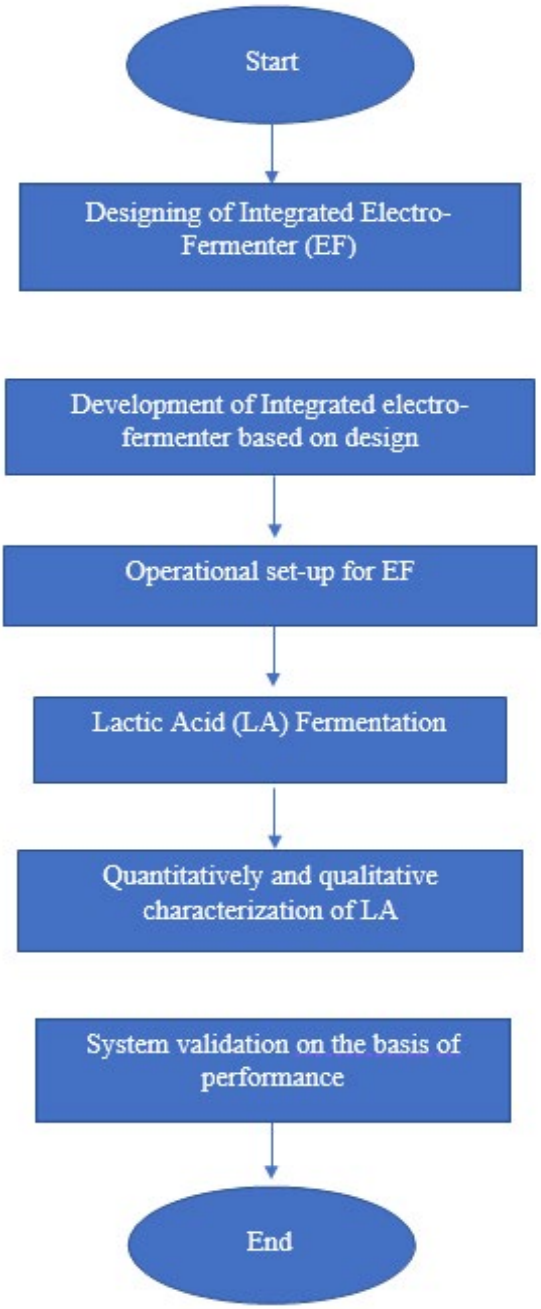

Figure 3.1 Methodology flowchart

3.4 Food waste sampling

The ability of the indigenous bacteria in converting substrates into lactic acid is measured quantitatively by determining the concentrations of lactic acid accumulated in the fermented food waste media. In this section, the food waste is homogenised into semi-solid consistency and fermented accordingly. 500 g of food waste was mixed with 1000 ml of deionized distilled water and homogenised in a food blender (modified from methods of Ohkouchi & Inoue, 2007).

3.4.1 Starter Culture

Yakult (*Lacticaseibacillus casei*) was used as starter culture. 100 ml was added into fermentative medium to assist in the start of fermentation by causing changes in the chemical composition of the process as well as the qualities of the substrate, resulting in a more homogenous product.

3.4.2 Isolation of lactic acid producing strain by autoclave high pressure steam

Autoclavation of the blended food waste is carried under temperature of 121 °C for about 15 minutes by using saturated steam under at least 100 kPa of pressure. Check the vessel and make sure the vessel is emptied and free from any possible inhibitor. Plenty of water enough to suffice the entire process is the put inside the vessel/chamber. Put the food waste that need to be sterilized inside the vessel and make sure to tighten all the screws to prevent any leaking and the electric heater is switched on. Safety valves are adjusted to keep the necessary pressure inside the vessel.

Once the water starts to be boiled up, the vapor is allowed to break free through the discharge tube and the complete displacement can be secured once the bubbles cease to come out from the pipe. The pipe is then closed and the steam inside the vessel are allowed to reach the desired level which is 100 kPa. The excess pressure removed after the desired pressure is reached if the whistle blows.

After the excess pressure removed, let the autoclave run for a period of 15 minutes to complete the process. The switch is turned off and let the autoclave to cool down until the pressure inside the vessel lowered than atmospheric pressure. The pipe is then opened to allow the air flow from outside into the autoclave. Lastly, lid is opened, and the sterilized materials are taken out of the chamber. (Cappucino JG, Sherman N, 1996) (JM, 2000) (GJ, 1989).

3.4.3 Fermentation of Food Waste

The pH of the and slurry were determined using a pH metre (HACH, USA) and then adjusted to pH 7 using disposable pipettes and 1 N sodium hydroxide. After the process of sterilization by autoclaving, starter culture was added to the slurry and being fermented in a 500 mL bottle in the laboratory for fifteen days at a room temperature which around 36.4^{o}C.

3.5 Parameter testing

100 ml of the slurry was taken to test the process parameter which is Biochemical Oxygen Demand (BOD), initial Ph and Chemical Oxygen Demand (COD)

3.5.1 Biochemical Oxygen Demand (BOD)

Prepared the solution which are 3L of distilled water and BOD nutrient buffer pillow for 1 capsule. The mixture solution was stirred until dissolve well using a glass rod. The food waste was checked using pH indicator. 8ml of food waste was taken and put into the 300ml BOD bottle using micropipette. The dilution of BOD nutrient solution was poured into the BOD bottle at inner surface that contained food waste solution to prevent from air bubbles.

Probe was used to measure the dissolved oxygen concentration in each bottle. The food waste was calibrated using the dissolve oxygen food waste and the mixture was stirred. The dissolved oxygen showed the result of the food waste and must be taken before the final result which is 5 days period of time.

Rinse the probe using distilled water and wipe using a tissue paper and kept in their solution. Carefully insert the stopper of BOD bottles to prevent air trapped inside and dilution water was put above the stopper of the BOD bottles to make a water seal. The BOD bottles covered with aluminium foil to prevent evaporation and kept into the incubator at 20ºC and left for 5 days.

3.5.2 Initial Ph

The effect of initial pH was conducted by adjusting starting pH in food waste slurry previously prepared in 3.4 with addition of 1 M natrium hydroxide and 1 M sulphuric acid using disposable pipettes

3.5.3 Chemical Oxygen Demand (COD)

COD digestion reactor were set up in 150º C. 2ml food waste was added into the homogeneous reagent (potassium dichromate) using the volumetric pipette 2ml. The mixture was mixed well and poured into the COD vial. Another COD vial were used and 2ml of distilled water is added and labelled as blank. COD vial with food waste was placed into the COD digestion reactor for heating purposed for 120 minutes. Reactor turned off and wait around 20 minutes until 120 ºC and put the vials outside on the rack, cooled down by room temperature.

The vials were inverted for several times during warmed and placed on the COD rack. Spectrophotometer was ON, setting and the program of 430 COD LR was selected then start button was pressed. The outside of the vials was cleaned using towel before putting into the spectrophotometer to avoid fingerprint or other mark. The blank vial was put into the spectrophotometer first for calibrating the reading. Then, with next vials that contained food waste and the reading for the range of the pollutant was taken and tabulated.

3.6 Lactic acid content determination by High-Performance Liquid Chromatography (HPLC)

Lactic acid was determined using the High-Performance Liquid Chromatography. The standard curve was constructed using different concentrations of lactic acid (1-5 mol/mol) prepared from concentrated liquid lactic acid (88-90 %) and double distilled water or deionized distilled water. Double distilled water is very important for this analysis and sample preparations in order to avoid any interference of metal ions that exist in regular tap water and distilled water that could affect colour formation. All preparations were done in 50 ml volumetric flasks. The stock lactic acid was prepared.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Introduction

This section explains the research framework. Materials, experimental setup, inoculum, analytical methods have been demonstrated clearly. The details of the experimental outcome have been discussed further in the relevant chapters. Figure 3.1 elaborates the research framework to provide a crystal-clear view on the experimental strategy.

4.2 Parameter Testing for Food Waste Treatment

After fifteen days of fermentation, parameter testing (COD, BOD and pH) was carried out to learn the efficiency of the food waste treatment. The result of day-zero is shown in Table 4.1. Table 4.2 show the result at day-fifteen of the fermentation process. White bread waste (BW) shows the decrease of 94% in COD reading while fruit waste (FW) decreases in 92%. For BOD result, BW shows the decrease of 77.9% while FW decreases in 80%. The reading shows that the removal of the organic and inorganic contaminant is being done while fermentation process occurs. The initial pH which in neutral phase pH 7 dropped to pH 4 after fifteen days of fermentation. During fermentation, the pH continues to be dropped as there is the presence of lactic acid.

Sample	pH	BOD	COD
A (BW)	7.10	650	20416
B (FW)	7.14	251	17043

Table 4.1 pH, BOD and COD result at day-zero

Sample	pH	BOD	COD
A (BW)	7.10	184	1276
B (FW)	7.14	61.3	1311

Table 4.2 pH, BOD and COD result at day fifteen of fermentation

4.3 Lactic Acid Content Determination

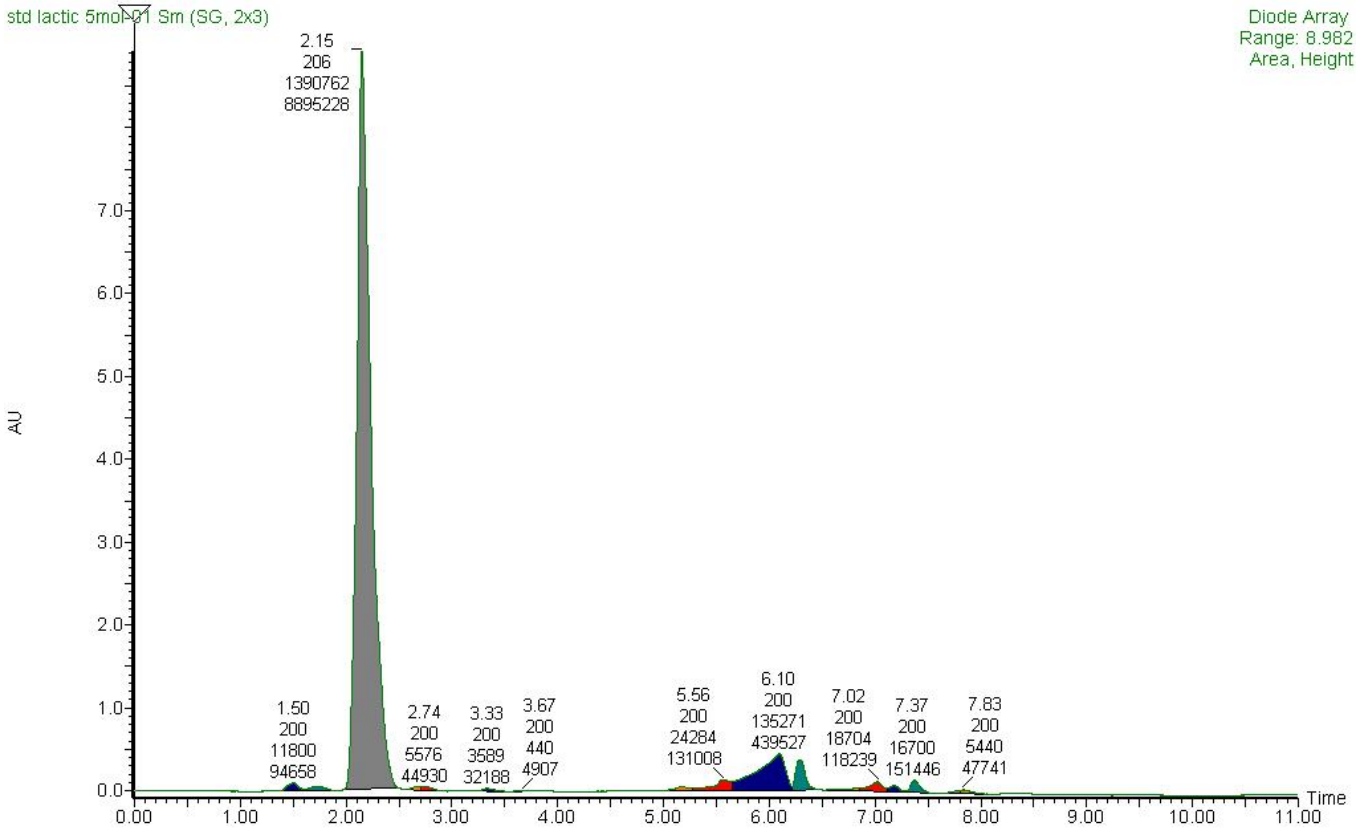

Figure 4.1 Standard solution of lactic acid qualitative for 5mol

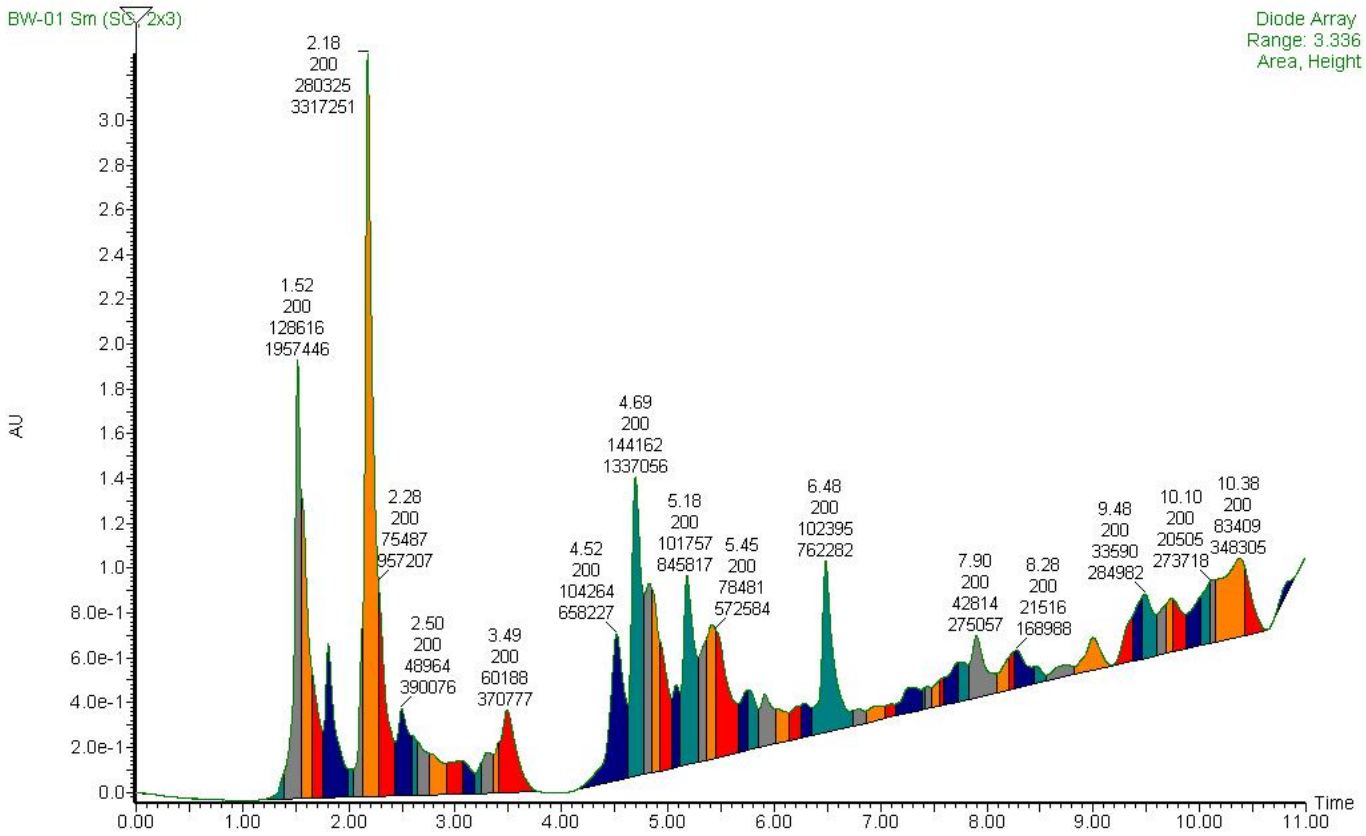

Figure 4.2 Lactic acid qualitative result for BW

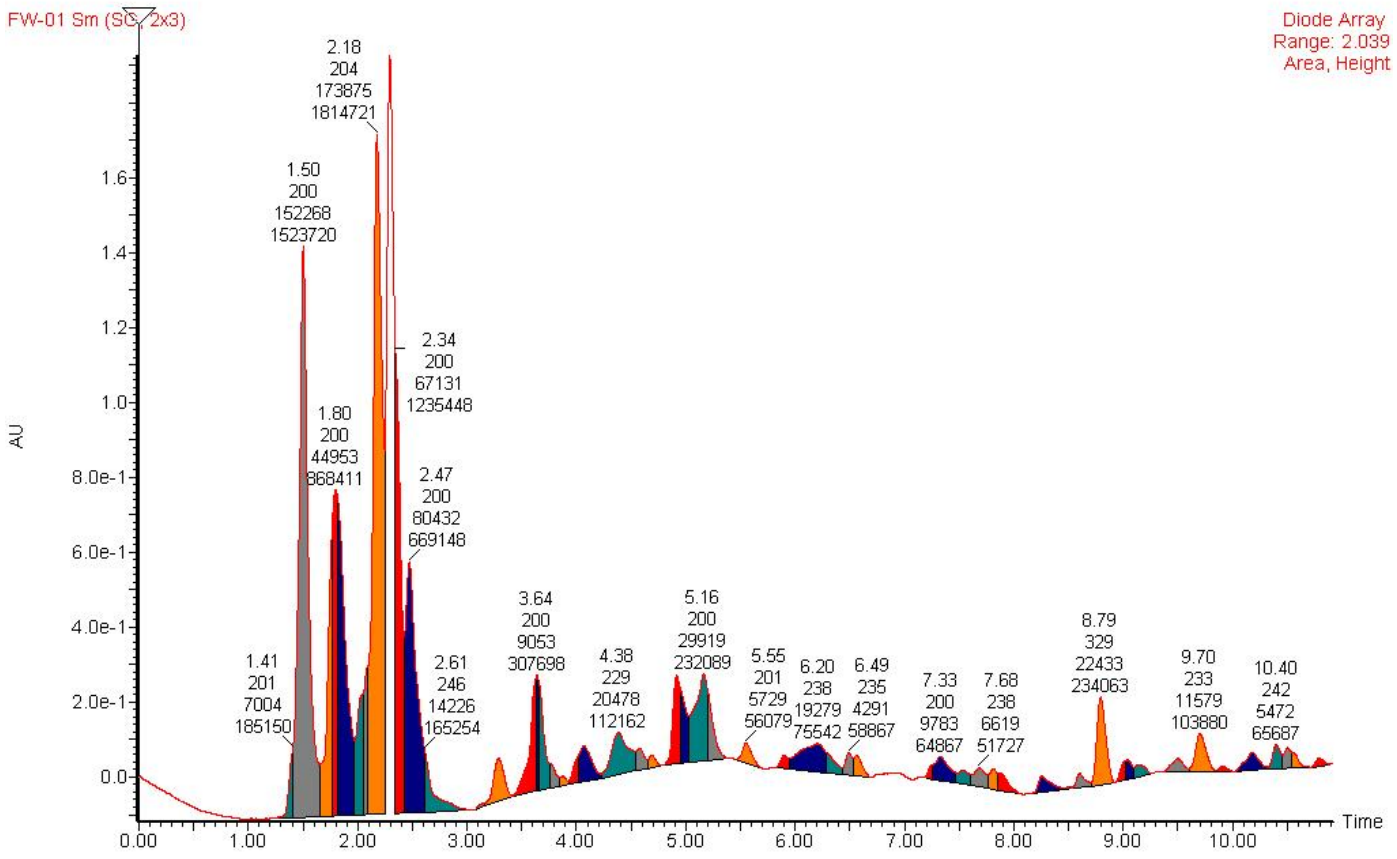

Figure 4.3 Lactic acid qualitative result for FW

Figure 4.1 showed the standard qualitative result for lactic acid. The retention time for the standard solution is 2.15 min (Table 4.3) with total volume area of 12.737 mm^3. In figure 4.2 and figure 4.3 are plotted the chromatograms of BW and FW at the end of the process (after fifteen days) and shows that BW with *Lactobacillus casei* cumulated the maximum lactic acid formation with total volume area of 0.929 mm^3 with retention time of 2.18 min while FW with *Lactobacillus casei* cumulated the maximum lactic acid formation with total volume area of 0.316 mm^3 with retention time of 2.18 min respectively. Both fig. 4.2 and 4.3 show the presence of lactic acid showed that the fermentation is taking place.

Sample	Ret. Time	Area	Height	Total Volume
Standard lactic acid	2.15	1.391	8.895	12.373
A (BW)	2.18	0.280	3.317	0.929
B (FW)	2.18	0.174	1.815	0.316

Table 4.3 High Performance Liquid Chromatographic result

CHAPTER 5

CONCLUSION

5.1 Conclusion

In conclusion, comparison of collected food waste shows that the collected food waste is as potential as waste to be used in lactic acid production. The important key components such as total sugar, carbohydrates and proteins. The starchy fraction of food waste namely white bread waste (BW) produced the most lactic acid yield compared to cellulosic fraction. The fermentation condition (duration, temperature and initial pH) for closed fermentation of food waste are 15 days, 36.4ºC and pH 7, respectively. At low initial pH, no lactic acid is produced but as initial pH becomes more alkaline, more lactic acid is produced. Highest lactic acid yield is obtained at initial pH 9. Total sugar is not affected by varying initial ph. HPLC results, illustrated that white bread waste has a higher lactic acid concentration when comparing the total volume to fruit waste (after fifteen days fermentation with addition of *Lactobacillus casei* at room temperature around 36.4°C).

5.2 Recommendation

Production of lactic acid from fermentation of food waste is a very promising method in waste management. The crude lactic acid could be used in production of cleaning agents, converted into technical grade lactic acid or even further purified to high purity lactic acid which is useful in production of polylactic acid polymer (PLA).

According to the outcomes of the study, the following recommendation may be suggested:

The comprehension on the fermentative capability of food waste can be further explored through microbial colonies evaluation of the waste and quantitative determination of the colonies. In industry scale, an open fermentation can be carry out because the cost is low and overall production of closed-fermentation can be pretty expensive.

Higher lactic acid quantity can be produced by co-cultures than the singly utilised strains. This could be inferred to be due to the joint action of the individual organism in the mixed culture, being able to overcome the nutritional limitations of utilised substrate. *Lactobacilli* co-cultures produced higher quantities of lactic acid than singly utilised starter cultures. Therefore, the combination of multiple starter cultures could be employed to improve yield of lactate during fermentation.

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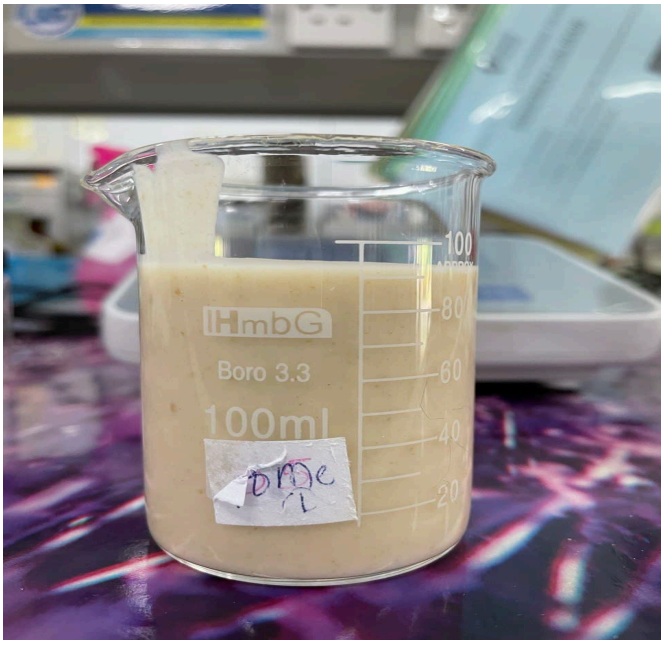
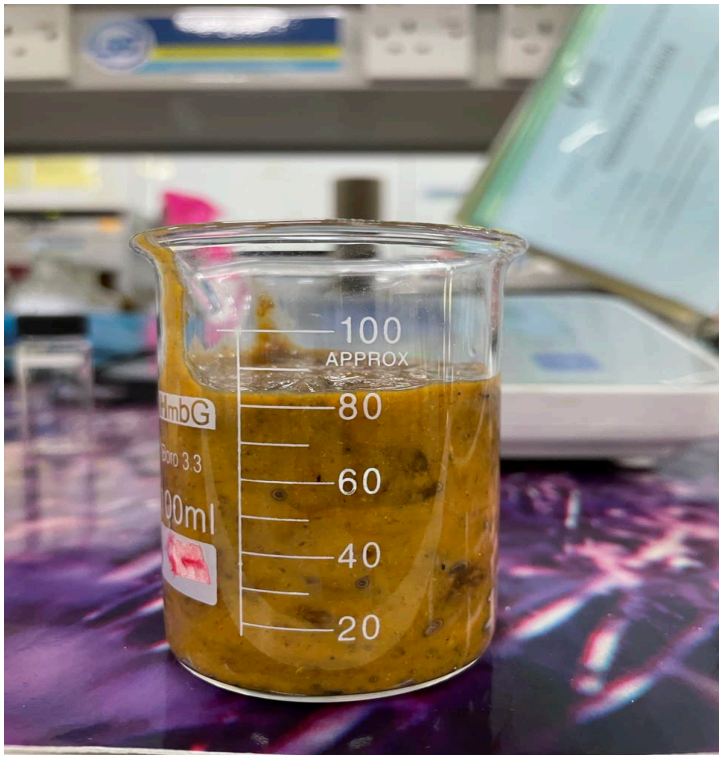
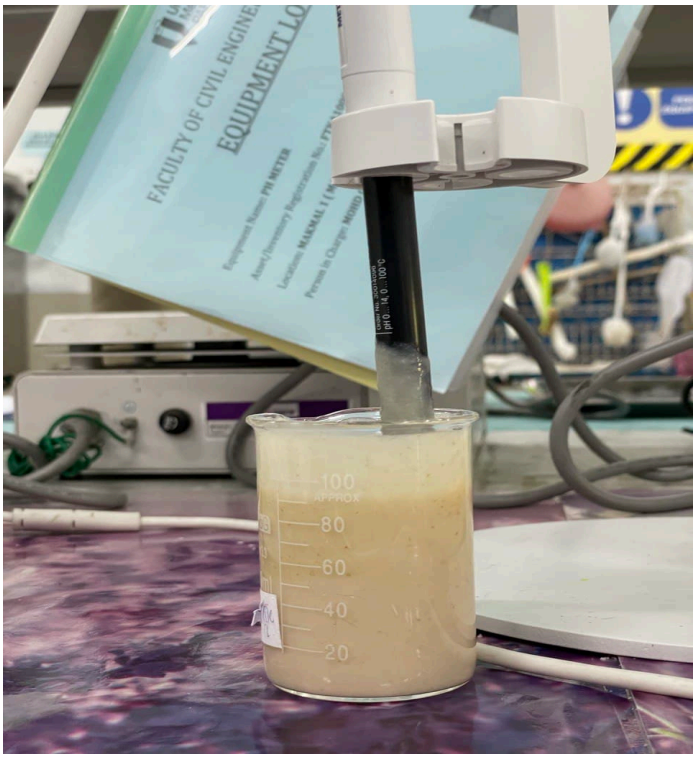
APPENDICES

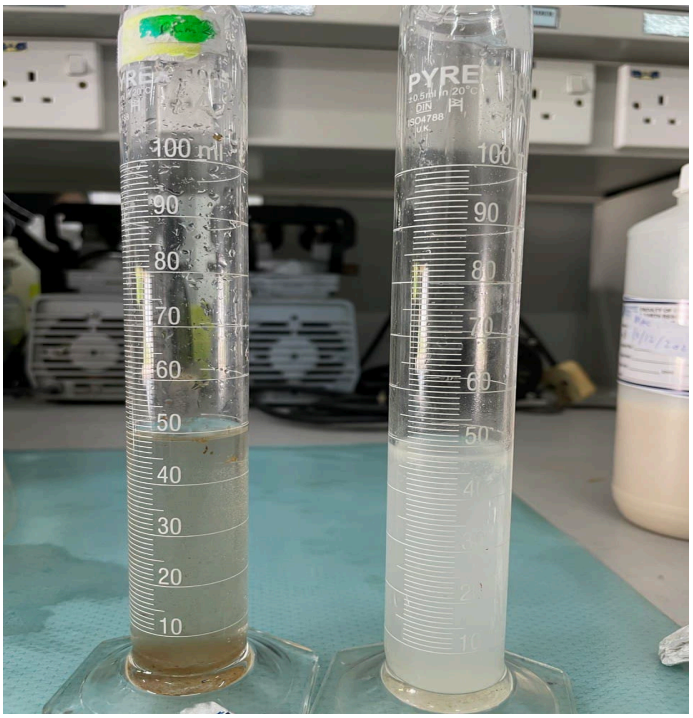
Appendix A: Sample Preparation





Appendix B: Parameter Testing



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ON
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HI839800
COD REACTOR
TIMER (min)
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