

DEVELOPMENT OF INTEGRATIVE FOOD
WASTE TREATMENT AND LACTIC ACID
PRODUCTION

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DEVELOPMENT OF INTEGRATIVE FOOD WASTE TREATMENT
AND LACTIC ACID PRODUCTION

NURSYAFAWATI BINTI BAKHARI

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ABSTRAK

Sisa makanan telah dibuang di tapak pelupusan sampah dan telah menyebabkan pelepasan gas rumah hijau, bau busuk, larut lesap toksik dan kutu kutu serta memenuhi kawasan tanah yang berpotensi. Di Malaysia, pengeluaran sisa berjumlah 45% yang mana 17,000 tan sisa makanan dikumpul setiap tahun dan dianggarkan meningkat kepada 3.18 juta tan pada tahun 2022. Berkaitan dengan penyelidikan ini, objektif yang dicapai adalah untuk melaksanakan ujian pengesanan ke atas proses rawatan sisa makanan dan untuk membangunkan penghasilan asid laktik daripada sisa makanan menggunakan proses penapaian. Selaras dengan peningkatan jumlah pengeluaran sisa makanan dan kesan merosakkan, cara yang lebih baik untuk menguruskan sisa organik ini diperkenalkan. Dalam penyelidikan ini, proses penapaian telah digunakan sebagai proses rawatan untuk mengitar semula sisa makanan bagi mencapai objektif. Terdapat dua jenis sisa makanan telah digunakan untuk penyelidikan ini iaitu sisa pertanian dan sisa domestik. Semua sisa makanan dikisar secara berasingan dalam 1L air dengan 500 g sisa makanan untuk membentuk buburan sisa makanan (termasuk pensterilan), pH awal diselaraskan kepada pH 7 dan ditapai secara statik pada suhu bilik 28 ° C. Selain itu, sisa makanan parameter diukur sebelum dan selepas proses penapaian untuk mengetahui kawalan sampel dan pecahan sisa makanan yang boleh menghasilkan hasil tertinggi ditentukan. Juga kecekapan sisa makanan, dari segi menghasilkan produk akhir iaitu asid laktik juga telah dikaji. Selain itu, sisa makanan yang paling berkesan untuk menghasilkan hasil rawatan sisa makanan yang tinggi kepada asid laktik ialah sisa domestik. Kira-kira 60-70% rawatan sisa makanan dicapai apabila 100 peratus nisbah vitagen yang digunakan sebagai inokulum dimasukkan ke dalam sampel sisa makanan 1 dan 2 untuk penapaian (proses rawatan). Hasil kajian menunjukkan kecekapan rawatan sisa makanan menggunakan proses penapaian bukan sahaja dapat menghasilkan hasil yang tinggi dalam proses rawatan makanan, malah dapat meningkatkan penghasilan asid laktik.

ABSTRACT

Food waste has disposed in landfills and has been caused to greenhouse gas emissions, foul odors, poisonous leaching and flea attraction as well as filling a potential land area. In Malaysia, the production of waste amount to 45 % which 17,000 tonnes of food waste was collected each years and estimated increased to 3.18 million tonnes in 2022. Related to this research, the objective has been achieved were to perform validation test on treatment process of food waste and to develop lactic acid production from food waste using fermentation process. According to the rising amount of food waste production and damaging effects, the better way to managing this organic waste is being introduced. In this research, fermentation process has been used as a treatment process to recycle the food waste to achieve the objective. There are two types of food waste has been used for this research namely agriculture waste and domestic waste. All the food waste has grinded separately in 1L water with 500 g of food waste into form of food waste slurry (including sterilization), initial pH adjusted to pH 7 and fermented statically at room temperature 28°C. Moreover, the parameters of food waste have been measured before and after fermentation process to know the sample control and the fraction of food waste that can produce the highest yield was determined. Also food waste efficiency, in term of producing the final product which is lactic acid also has been studied. In addition, the most efficient food waste on produce the high yield of food waste treatment to lactic acid is domestic waste. About 60 – 70% of food waste treatment has been achieved when 100 percent ratio of vitagen used as inoculum was added into food waste sample 1 and 2 for fermentation (treatment process). The results showed that the efficiency of food waste treatment using fermentation process not only can produce high yield of food treatment process, also can enhanced lactic acid production.

TABLE OF CONTENT

DECLARATION	i
TITLE PAGE	i
ACKNOWLEDGEMENTS	ii
ABSTRAK	iii
ABSTRACT	iv
TABLE OF CONTENT	v
LIST OF TABLES	ix
LIST OF FIGURES	x
LIST OF SIMBOLS	xi
APPENDIX	xii
CHAPTER 1	1
INTRODUCTION	1
1.1 Introduction	1
1.2 Problem Statement	3
1.3 Specific Objectives	4
1.4 Project Scope	4
1.5 Importance of Study	4
CHAPTER 2	5
LITERATURE REVIEW	5
2.1 Production of Food Waste	5
2.2 Food Waste Generation	6
2.3 Characteristic of Food Waste	8

2.4	Economical Potential of Value Added Products from Food Waste	9
2.5	Historical of Lactic acid	10
2.6	Physical Characteristic	11
2.7	Chemical Characteristic	12
2.8	Lactic Acid Production from Food waste	13
2.9	Application of Lactic Acid	13
2.9.1	Poly – lactic acid polymer	14
2.9.2	Pharmaceutical	15
2.10	Lactic acid fermentation	16
2.11	Uses of lactic acid	17
2.12	Importance of lactic acid	18
2.13	Fermentation via Lactobacillus Bacteria	19
2.13.1	Fermentation Operating Condition and Parameter	21
2.13.2	Microbial strain	22
2.13.3	Effect of temperature	22
2.13.4	Effect on pH	23
2.14	Fermentation mode	23
2.15	Continuous fermentation	24
2.16	Substrate of lactic acid production via fermentation	25
2.17	Lactic acid producing microorganisms	26
2.18	Types of microorganisms	27
2.19	Autoclaving	27
2.19.1	Autoclaving Process	28
2.19.2	Autoclaving procedure	29
2.19.3	Autoclaving in term of food waste	30
	CHAPTER 3	31

METHODOLOGY	31
Introduction	31
3.1 Material and Methods	33
3.2.1 Chemicals	33
3.2.2 Strain	33
3.2.3 Food Waste Source	33
3.2 Experimental Methods	34
3.3.1 Preparation of Food Waste	34
3.3.2 Inoculums Preparation	34
3.3.3 Autoclave	34
3.3 Fermentation of food waste	36
3.4.1 Chemical composition of food waste	36
3.4 High – performance liquid chromatography	37
3.5 Methodology flowchart	38
3.6 Analytical Methods	39
3.6.1 pH	39
3.6.2 BOD	39
3.6.3 COD	40
CHAPTER 4	41
RESULTS AND DISCUSSION	41
4.1 Introduction	41
4.2 Fermentation Process	42
4.3 Performance Analysis	43
4.3.1 BOD different before and after fermentation process	43
4.3.2 COD different before and after fermentation process	44
4.4 Effectiveness (%)	45

4.4.1	Percentage (%) of BOD at 28°C before and after fermentation process	45
4.4.1	Percentage (%) of COD at 28°C before and after fermentation process	46
4.5	Lactic acid stock solution calculation	48
4.6	Lactic acid result using HPLC	50
4.6.1	Standard Lactic Acid 5mol	50
4.6.2	Lactic acid observation in sample A	51
4.6.3	Lactic acid observation in sample B	52
CHAPTER 5		54
CONCLUSIONS AND FUTURE SUGGESTIONS		54
5.1	Conclusions	54
5.2	Recommendations for Future Studies	55
REFERENCES		56

LIST OF TABLES

Table 2.1	Physical Composition in food waste	11
Table 2.2	Chemical Composition in food waste	12
Table 2.3	Field of application and the benefit that contribute for lactic acid production.	14
Table 2.4	Secondary metabolites produced by LAB compatible with the multi – product bioprocess (Jise Anibal Mora - Villalobos, 2020)	20
Table 2.5	Lactic acid isomer produced by Lactobacillus species	21
Table 2.6	Summary of the substrate for lactic acid fermentation ((Bintsis, 2018)	25
Table 4.1	BOD result at 28 °C before fermentation process	43
Table 4.2	BOD result at 28 °C after fermentation process	44
Table 4.3	COD result at 28 °C before fermentation process	44
Table 4.4	COD result at 28 °C after fermentation process	44
Table 4.5	Lactic acid standard	50
Table 4.6	Lactic acid value in sample A	51
Table 4.7	Lactic acid value in sample B	52

LIST OF FIGURES

Figure 2.1	Poverty Pollution of food waste production in Malaysia (SWCorp, 2019)	6
Figure 2.2	Food waste pollution in Malaysia (SWCorp, 2019)	7
Figure 2.3	Sustainable Development to halve food waste	9
Figure 2.4	Demand for lactic acid (research, 2021)	17
Figure 2.5	Lactobacillus casei	19
Figure 2.6	A basic Autoclave process (Rogoff, 2018)	29
Figure 3.1	Schematic diagram summarizing the experimental methodology	32
Figure 3.2	Autoclave set - up	35
Figure 3.3	The sample after sterilization	36
Figure 3.4	The BOD result for sample A and B after 5 days	40
Figure 4.1	Reactor of fermentation process	42
Figure 4.2	Percentage (%) of BOD at 28°C before and after fermentation process	45
Figure 4.3	Percentage (%) of COD at 28°C before and after fermentation process	46
Figure 4.4	Determination of lactic acid in standard time vs standard	50
Figure 4.5	Determination of lactic acid in sample A	51
Figure 4.6	Determination of lactic acid in sample B	52

LIST OF SYMBOLS

$^{\circ}\text{C}$	Degree Celcius
%	Percentage
$\text{C}_3\text{H}_6\text{O}_3$	Chemical formula organic compound
CHNSO	Analysis for organic and inorganic samples
PLA	Poly – lactic acid
LA	Lactic acid
AHA	Alpha hydroxyl acid
MT	Metric tons
LAB	Lactic acid bacteria
N_1	Assumed amount of stock solution
V_1	Unknown value of double distilled water (x)
N_2	Volume of stock solution
V_2	Volume of double distilled water

LIST OF APPENDICES

Appendix A: Fermentation process of food waste	61
Appendix B: Lactic acid solution preparation	62
Appendix C: Gantt Chart SDP 1	63
Appendix D: Gantt Chart SDP 2	64
Appendix E: Cost Project	65

CHAPTER 1

INTRODUCTION

1.1 Introduction

Food waste is a global issue that is growing fast as a result of food security concerns and related into environmental. Food waste give serious impact into food industry, environment and the economy (Effie papargyropoulou, 2019) Furthermore, food waste contributed 23% in economic industry of the food purchased. Food waste has a high energy potential, biodegradability and significant energy recovery.

According to this study, the best way to reduce amount of food waste is avoiding to throwing out any food everywhere. According to (Paul van der werf h. A., 2019), this issues give a negative attitude toward environment. Integrative techniques have proven for maximization of energy recovery from food waste including to perform the validation test on treatment process of food waste. According to FAO (Food and Agriculture Organization) reported in 2017, shows that food waste nationally accounts for one – third of the total food produced for human consumption around 1.6 billion tonnes each year.

Furthermore, to minimize the production of food waste in Malaysia, the alternative way has been introduced. This alternative way is easy process to reduce food waste production around the world also no need the high cost to implement it in real life. According to (Fabian Bonk, 2017), said, over the last few years, alternative technologies such as the fermentation process have been developed to produce value – added products from food waste. Moreover, food waste contains a rich substrate for the production of lactic acid via sterilized food waste with additional nutrient and continuous pH adjustment to produce high yield of lactic acid production.

(Ali Nawaz Q. M., 2017) said, lactic acid is used in a variety of industries including cosmetics, pharmaceuticals, chemicals, food and medicine. Moreover, lactic acid can be produced through a variety of processes including fermentation of sugars and food waste. Lactic acid can produce chemical through enzymatic catalysis and fermentation. Due to the availability of raw material, 90% of lactic acid is produced by microbial fermentation (Liang Song, 2021). Fermentation is a microbial fermentation process in which microorganisms break down organic molecules to obtain energy.

Lactobacillus are widely used as strains rather than yeast or other microorganisms and occurs widely in nature and fertilizes (Liang Song, 2021). Furthermore, lactic acid bacteria can generate electricity from waste through organic matter such as added nutrient to develop electron, proton and carbon dioxide. Microbial fuel cell (MFC) one of alternative technique approach to transform waste become energy saving which is MFC is provide low cost alternative to conventional.

In this research, to develop integrative food waste and lactic acid production by using the fermentation process. Compare to the conventional process which is hydrolysis, usually use commercial enzymes and at the same time can produce a large amount of food waste using different equipment. But this process can be costly. To have effectiveness result, the microbe (Lactobacillus) will be added into the food waste to prepare the medium for the bacteria. Furthermore, in this research lactic acid also can be used to produce the electricity. The diverse applications of lactic acid, combined with the growing demand for environmentally products (Tsz Him Kwan, 2017).

1.2 Problem Statement

Food waste can be determined as food consumed by humans and can be generate at any level of the food chain including by – product or waste product. Recently, food waste has become a complex matter that has attracted the attention from researcher and scientists around the world (Rinkesh, 2020). Food waste is a never – ending problem in Malaysia, with households account for (Zainal, 2021), around 45 % which 17,000 tonnes waste comes from food waste that becomes produced every years. To support this statement, eating spoiled food will be result in severe health problem also cost increase.

Moreover, food waste can cause environmental issues which is climate change into the world such as, will produce a greenhouse gasses even more potent that carbon dioxide. In addition, greenhouse gas emission can be reduce between 6% - 8% if people can stop wasting food (Fund, 2020). This is needed the alternative ways to reduce the waste production in order to save the environment also world health. Plus, this will be the great impact into economic and social which food waste is defined by the BFCN (Barilla Center for Food and Nutrition) as food waste or loss that occurs during industrial activity, transportation and use (Fund, 2020).

Lactic acid production can be optimize through recycling of value - added products and waste reduction (Jialing Tang, 2016). In this research, the main focus is to develop the new alternative to perform validation test using food waste and to determine lactic acid inside the waste which is available and easy to conduct experiment. Lactic acid industrial is important chemical platform which is used a pH regulator and preservative in the industries (C. Rodrigues, 2017). Furthermore, lactic acid demand was estimated can be reach 1,947 kilo tonnes in 2019 and it expected to increase between 16.2% per year started in year 2019 to 2025 due to increase production in medicines and perfumes (W, 2021). Therefore, due to produce lactic acid production more effectively into industry, the new alternative method has been introduced which implementation of fermentation process in food waste. This method is more complex and does need high cost to implement into industry.

1.3 Specific Objectives

There are two objectives from this project which are:

- i. To perform validation test on treatment process of food waste.
- ii. To develop lactic acid production from food waste using fermentation process.

1.4 Project Scope

This research is focusing to perform the validation test on treatment process of food waste and to develop lactic acid production from food waste using fermentation process. The selected area for this study at cafeteria UMP Gombang. The food waste was collected from KK3 cafeteria and have been store before weighed according the composition that has been made. This study only covered one cafeteria where situated near the hostel building. This research has focusing to determine lactic acid production using different waste to prove that the efficiency treatment food waste that can produce product. This study measured physical characteristic on pH and temperature of the food waste. Meanwhile, the chemical characteristics covered on the nutrient contents of food waste such as biochemical oxygen demand (BOD) and chemical oxygen demand (COD). The data obtained was used as references in determining the potential of produce lactic acid from food waste. High Performance Liquid Chromotography (HPLC) are being use to observe the lactic acid production.

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

This chapter will go through about the background of food waste industry, types of treatment process, lactic acid production, application of lactic acid and types of microorganisms. Immobilized treatment process is used for the production of lactic acid. More than half of total lactic acid consumption is conventionally produced in low productivity simple group fermentation. Basically, this chapter will be synchronise with the objective related to this experiment.

2.1 Production of Food Waste

Food waste can be classified as any food wasting around world. In other words, food waste is defined as unwanted raw or cooked food that is discarded during or after food preparation. It is no longer fit for consumption or desirable. The example of food waste including, contain multiple types which is meat, noodles, vegetables, rice, fruits, bread and also potatoes (Daniel Pleissner F. D., 2017).

Several options are convenient for managing food waste such as composting, incinerating and disposal to land (landfilling). Accordingly, landfilling is the most popular and suitable method in Malaysia for dispose of food waste and other solid waste. However, this method is insufficient to support the increasing amount of food waste. Furthermore, uncontrolled food waste may cause environmental issues and health impact to human.

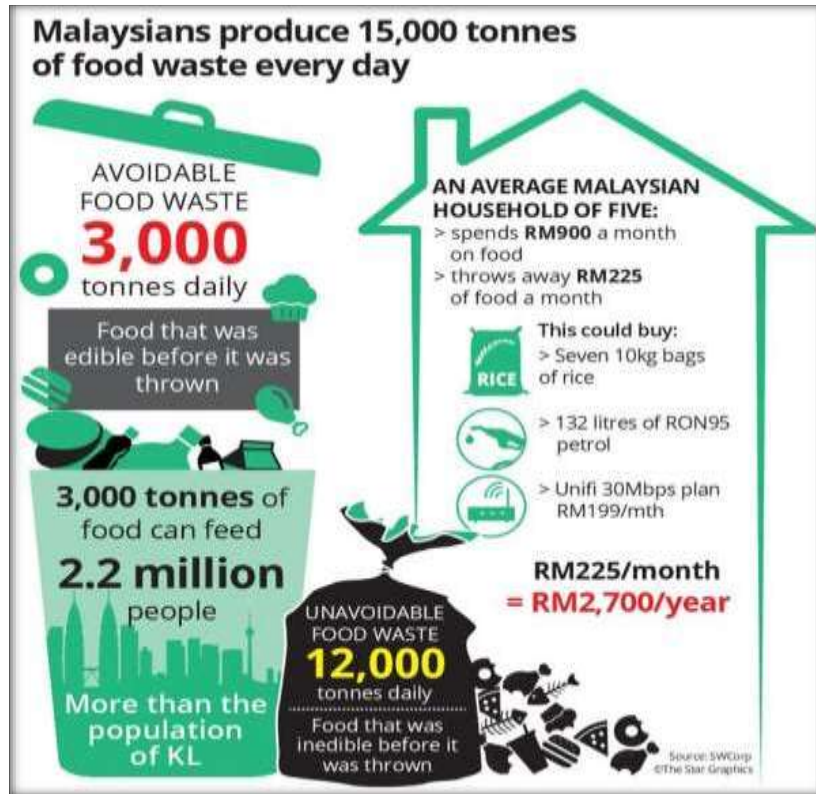


Figure 2.1: Poverty Pollution of food waste production in Malaysia (SWCorp, 2019)

2.2 Food Waste Generation

In recent years, food waste (FW) generation has increased dramatically according to economic and population development. The global, environmental and societal effects of food waste are prompting researchers and scientists to think of new ways to fix the issues associated to food waste disposal (Ashfaq Ahmad, 2021). Food waste will effect and occurs at all stages between production and consumption. Nationally, production and storage account for 54% of unwanted waste, while processing, distribution and consumption counted for the rest. There is no solution to reduce the food waste, hence the upcycle method unless produce more valuable products from waste.

One – third of all food produced for human consumption is lost or wasted each year, counteracting over 1.3 billion tons a year. Therefore, it will be the biggest cause of food wastage at any level between production and final disposal. Contrast with residual flow from relatively consistent food harvest and processing, consumer wastes is made up of a different element changed according to seasons and location.

According to a sustainable UN development, their objective related for responsible consumption and production urge the world to “half per capita global food waste at the retail and consumer levels and reduce food losses throughout production and supply chain, including post – harvest losses“ by 2030 (Effie Papargyropoulou, 2019). Food waste is the third largest fraction of municipal food waste (MFW) contributed 12.5% from other waste. Furthermore, many household waste contribute along, with 14.7 million populations in developed countries (Food and Agriculture United Nations, International Fund for Agriculture Development & World Food Program, 2015) (Paul van der werf J. A., 2019).



Figure 2.2: Food waste pollution in Malaysia (SWCorp, 2019)

2.3 Characteristic of Food Waste

Food waste has been increase and effect the global economic population now on days. Usually, food waste was produced by lodgings, restaurants, households, canteens and commercial building or property. A developing country such as Malaysia has recorded a generation of municipal solid waste (MSW) around 38,000 t/d in 2018. According to (Tsz Him Kwan, 2017), approximately 300,000 to 400,000 metric tonnes (MT) per year of food waste with industrial application grow at a rate of 18.6 percent per year. Majority of food wastes are characterized as easily degradable, high moisture content, low pH and highly solubility. It provides more energy per day mass. Characteristic of food waste can be measured by physical and chemical contain in the food waste.

Food waste is referred as raw material which is food waste are used to identify waste which started in specific place or location such as canteen at the university. Furthermore, there are various benefits to food waste as raw material such as low handling cost due to minimum collection and transportation cost and high biodegradability. Food waste may contain few sources depending on variety types which is meat, noodles, vegetables, rice, fruits, bread and also potatoes (Daniel Pleissner F. D., 2017).

In addition, food waste is used as the only substrate at a low organic loading rate (OLR) which related with process complication such as acid accumulation, excess inhibitory, component concentration and nutrient contrast. According to (Izhar, 2021), comparing to fruit vegetable waste, food waste has a lower C/N ration and pH which fruit and vegetable waste contain high lignocellulose waste and peelings.

2.4 Economical Potential of Value Added Products from Food Waste

Agriculture production and agro – industrial processing produced a large amount of by – product and wastes. Fresh fruit by – product such as sugarcane waste, skin, pruning, steams, shells, bran and seeds comprise more that 50% of fresh fruit and also have a higher nutrient or functional content that the finished product (Cristian Torres Leon, 2018). Recycling food waste to lactic acid can bear higher economic potential than others suggested use of waste sources such as methane and ethanol fuels. Methane generation as accepted use of food waste (Merlin Alvarado - Morales, 2021).



Figure 2.3: Sustainable Development to halve food waste

2.5 Historical of Lactic acid

Lactic acid can be defined as (2-hydroxypropionic acid, $C_3H_6O_3$) is an organic hydroxyl acid which is found in naturally in nature. Lactic acid was discovered in 1780 by Swedish chemist Carl Wilhelm Scheele, and it was first commercially created in 1881 by Charles E. Avery in Littleton, Massachusetts. Furthermore, it was the first organic acid to be commercially created through fermentation started in year 1881 (Fatih Ozogul, 2021). Moreover, it can be found in variety foods naturally or as a result of microbial fermentation. It is also specific metabolic pathway in most living organisms from anaerobic prokaryotes to human.

In 1839, Fremy tested the lactic acid fermentation on various of carbohydrate, including sugar, milk sugar, mannite, starch and dextrans. Moreover, in 1840, Lourdou produced lactic acid through fermentation whey and transformed it into iron lactate by dissolving metallic iron in it. After 1847, a few different scientists have performed fermentation experiments to produce lactic acid from sugar cane. Blondeau defined lactic acid as a fermentation product by year 1847. Initially, lactic acid was fermented and identified in muscle tissue considered the same. Liebig believed that the two acids were never the same, when he re-examined meat extract.

In 1873, Welcenus observed that they have the same structure but different physical properties. Moreover, it was also researched by Pasteur as one of the first microbiological yeast culture distillery, but it was not until 1877 lactic acid bacteria were isolated in pure culture when Lister identified *Streptococcus lactic*. During the same time, Delbruck was searching the best temperature for lactic acid fermentation in a distillery. According to (Fatih Ozogul, 2021), reached the conclusion that the high temperature preferred yield of lactic acid output.

2.6 Physical Characteristic

The physical parameter of waste including particle size distribution, geometry and classification of the waste, moisture and organic matter content, unit weight and temperature of the landfilled waste.

One of the major method suitable to manage solid waste management which is composting. It provides means of producing a valuable end product by treating organic wastes in environmentally friendly procedures that does not released any hazardous chemical that can harm human health without causing serious disruption to the surrounding ecosystem. Furthermore, the issue of time consumption arises and this refers to the sink of market demand. According to (Fatemeh Hassan Pour, 2021), various method of disposing food waste and recycling (landfilling, composting, anaerobic digestion and incineration) were discussed.

Physical Composite (%)	
Rice	38.72
Bakery products	18.74
Meat	25.15
Fat	13.03
Bones	2.19
Fruits and vegetable	2.16

Table 2.1: Physical Composition in food waste

2.7 Chemical Characteristic

Food waste come from different sources or stages of yield. Furthermore, it contains different compositional characteristic and suitable for recycling into different products. To have better understanding of chemical parameter of food waste, the sample will be collect in different types of food waste. According to (Ho, 2018), characteristic and facilitate of food waste management, composition of food waste can be determined by collecting the waste from different sources.

The chemical parameter of waste including moisture content, volatile solid, ash content, CHNSO contents, calorific value and heavy metal. The chemical characteristic of food waste contains the value of pH which is can be between 6.15 to 6.17, based on the types of food waste used including with the chemical parameter was mention before.

Chemical Composition (%)	
Moisture	38.4
Carbohydrates	25.56
Crude Protein	17.26
Crude Fat	15.27
Fibber	0.3

Table 2.2: Chemical Composition in food waste

2.8 Lactic Acid Production from Food waste

Lactic acid is a precursor to multiple products such as in chemicals, textile, various medical, cosmetic, food and pharmaceutical industries (Sumit Kumar, 2020). Normally, lactic acid is produced through the fermentation of carbohydrates such as glucose, starch and molasses. The successful commercial of lactic acid depends on the effective lactate esters as alternatives petrochemical sources and provide low cost. To produce low cost production of lactic using must use the inexpensive and simply accessible substrates. Moreover, lactic acid can be appeared through chemically or biologically. According to (Andrea Komesu J. A., 2017), lactic acid is a precursor to multiple products such as in chemicals, textile, leather, cosmetic, food and pharmaceutical industries.

Food waste is highly substrate with readily degradable carbohydrates that could be ideal for acid production. In addition, lactic acid is the dominant product under the proper digestion conditions. Besides, lactic acid has a high value when compared to other acids. This is primarily due to the high demand for poly-lactic acid polymer production, but it also has inherent in secondary treatment works at wastewater treatment plants. The implementation of lactic acid in these market, can be the possibility of producing lactic acid production from food waste, are discussed in the following sections. Lactic acid has uses in applications in chemicals, pharmaceuticals, and it also a precursor to a few products.

2.9 Application of Lactic Acid

Lactic acid is usefully in food, pharmaceutical and technical grades. Lactic acid is a biodegradable organic acid useful (C. Rodrigues, 2017) in pharmaceutical, chemical and food industry. Moreover, lactic acid has been more important and used in the multiple of application, salts, esters and many of their derivatives have been produced. Uses of lactic acid can be categorized by grade and lactic acid derivatives. Some important applications of lactic acid are used in many industrial productions.

Field of application	Benefit/impacts of respiration
Starter culture	Higher cell count
Food products	Greater acetoin/diacetyl production for aroma
Biotechnology	Less stressful conditions for protein production
	Heme – inducible reporter and expression system
Health, diet	Vitamin production by some LAB
	Lower home toxicity in gut due to heme utilization
	Long – lusting probiotic cultures
Plant biology	Acetoin is a plant – signaling molecule that stimulates growth

Table 2.3: Field of application and the benefit that contribute for lactic acid production

2.9.1 Poly – lactic acid polymer

The main application of lactic acid is in production of polylactic acid (PLA) polymer. The production of lactic acid including three predefined which is direct condensation, ring opening polymerization and azeotropic distillation which route by lactic acid fabrication by PLA. Plus, direct condensation produces low molecular weight PLA chains with suboptimum features. Next, the ring opening polymerization route includes a direct condensation step, followed by the formation of lactate rings and it will produce high molecular weight chains. According to (Ashfaq Ahmad, 2021) lactic acid (LA) is a bioprocessing platform molecule with promising applications in food, chemicals, pharmaceuticals and biodegradable plastics. For all processes, lactic acid is rapidly produced of degraded digestion carbohydrates.

2.9.2 Pharmaceutical

Lactic acid is a major component in the pharmaceutical industries. Lactic acid has a wide range of industrial uses, which has contributed to increased concentration on lactic acid in multiple industries (Ali Nawaz, 2017). The pharmaceutical and food industries show the availability of lactic acid L (+) because the D (-) isomer is not digested by the human body. Lactic acid and its salts have been related of variety medical conditions. In addition, they generate energy and volume for blood in addition to pH regulation. New formulation was found in pharmaceutical industries uses for anti tumor activity such in calcium, sodium, ferrous and other lactic acid salts. Nowadays, lactic acid is also used in the pharmaceutical and cosmetic industry because of its function capabilities (Ramzi A. Abd. Alsaheb, 2015).

Biodegradable plastics have been produced from poly (lactic acid) have been evaluated for use as biodegradable implants for the healing of fracture and other injuries. This application can be divided into the following:

- Pharmaceutical/ medical
- Hair and skin care (cosmetics industries)
- Lactic acid (skin renewal)
- Sodium and ammonium – lactate (skin moisturizer)
- Hair conditioner

The calcium salt of lactic acid is produced in granular and powder form. Calcium lactate trihydrate is mainly used in pharmaceuticals as a calcium supplement and a blood clotting agent for use in the treatment of bleeding and to minimize bleeding during dental surgery. Furthermore, sodium lactate is used in the production of some antibiotic and medical formulation which is used to buffer pharmaceutical preparations.

Natural lactic acid L (+) is used in a variety in cosmetics. Lactic acid also knowns as alpha hydroxyl acid (AHA) and is found in the skin. Lactic acid and sodium lactate are widely used as deep moisturizing agents many skin care products since L (+) lactic acid is naturally found in skin. Besides, lactic acid also can be used to increase pH as a solution. It is one of the AHAs efficient with the lowest probability for irritation.

2.10 Lactic acid fermentation

Lactic acid (LA) is the process to produce bacteria, *Lactobacillus plantarum* MTCC 1407 and 6161 will produced using IMTECH chandigarh. Fermentation process is the chemical method which is has an advantage in produce optically pure L- lactic acid, since the latter produces a racemic mixture of DL-lactic acid (Sumit Kumar, 2020). The L form in lactic acid is useful in food and pharmaceutical application because human digest it. To perform the fermentation sterilization, must has the inoculum that can active the process which is *Lactobacillus*. This chemical function to breakdown the food and absorb the nutrient.

Therefore, calcium carbonate was used to extract the sugar and neutralized by using pre-treatment process. The supplementary nutrients (g/L) such as yeast extract (5.5), inoculum *Lactobacillus* were added in addition to lactic acid extract for growth medium preparation and pH was adjusted. *Lactobacillus plantarum* was proven to be effective producing lactic acid from OFIC350 (granulation 350 μm) hydro-lysate and OFIFP extract without detoxification (Besma Derabli, 2021).

The preparation for medium sterilized at 121°C for 15 min and cooled to room temperature. 70% substrate and 10% of inoculum with lactic acid bacteria was prepared and incubated at 35°C at 100rpm for 24 hours. According to (Emmanuel Alepu Odey Z. L., 2018), total time to remove microorganisms in sludge by fermented food waste takes between (7 – 14 weeks) compared with hydrated lime (about 1h) and urea treatment (about 4 days).

2.11 Uses of lactic acid

Lactic acid is used as a humectant or moisturizer in some cosmetics and a chemical that helps fabrics dyes in textiles. Moreover, it is also used in making sour food such as pickles and sauerkraut. Lactic acid also used in the dairy industry such as making yogurt and making cheese as well. Also, it is used in leather tanning. Lactic acid production is important product used 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. Although, lactic acid is used in the food industry for acid property in the production of soft drinks, candies, 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 and surgical sutures.

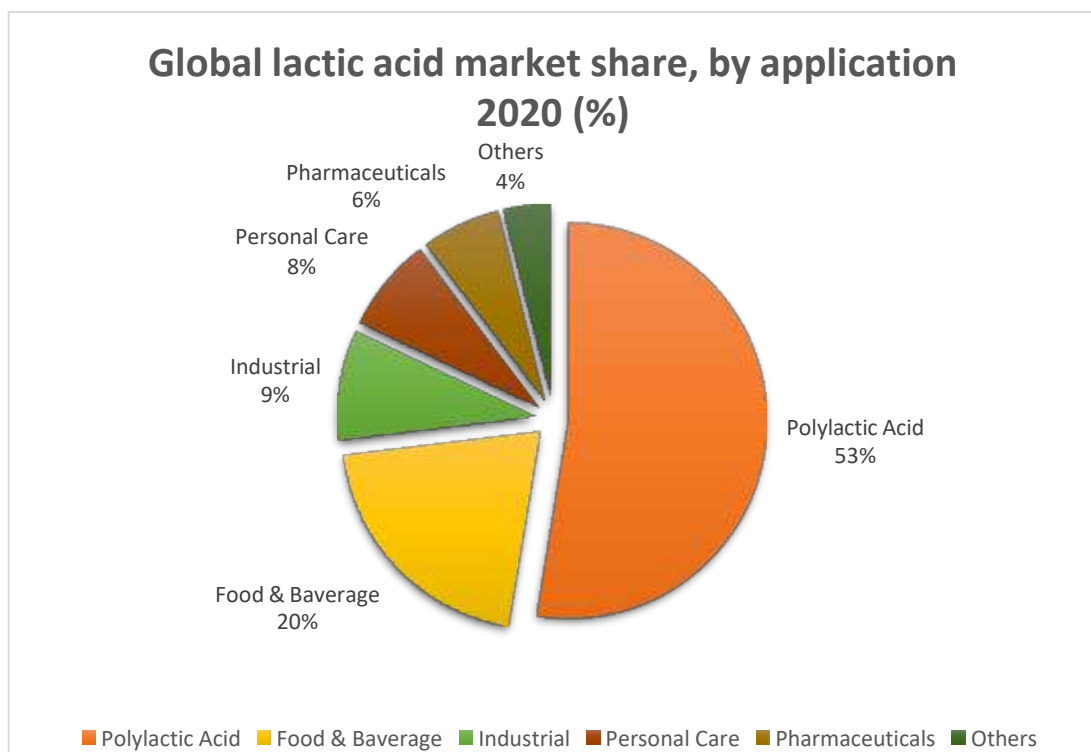


Figure 2.4: Demand for lactic acid (research, 2021)

2.12 Importance of lactic acid

The importance of lactic acid is widespread in the food industry, appear to be generally of certain life forms and particularly lactic acid bacteria. As a result, fermentation bacteria are widely applied in the food industry as a starting culture for food industry processing. Furthermore, lactic acid can be used as food additives in the edible products industry without the presence of lactic acid bacteria. This method can be useful in a variety of situations. (Emmanuel Alepu Odey B. O., 2018).

In addition, lactic acid is widely used in all branches of the food industry to regulate pH, provide aroma and improve the quality and flavor of foods (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 A. A., 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 lead 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 (Lactic 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.

2.13 Fermentation via *Lactobacillus* Bacteria

Lactobacillus species are probiotic bacteria (“good” bacteria) which are commonly found in the human digestive tract and urine. Furthermore, they are useful for digestion and “intestinal health”. According to (Linh Phuong Ta, 2021), the optimal ingestion dose of probiotics can vary depending on the strain. In addition, industrial fermentation uses multiple species of *Lactobacillus* because of its higher conversion, yield, and rate of metabolism.

Lactobacillus is usually found in substrates containing carbohydrates, such as plants and organic plant substances. In addition, homo-fermentative *Lactobacillus* cultures are believed to be the most important commercial species for producing lactic acid through fermentation. According to (Bintsis, 2018), lactic acid bacteria (LAB) are a vast mix of bacteria which play an important part in different fermentation processes. In general, the ability of a potential industrial *Lactobacillus* culture to efficiently and effectively convert a cheap substrate to L (+) lactic acid with the minimum amount of nitrogenous material supplement is required. Some bacterial strains, such as *Lactobacillus casei* and *actobacillus delbrueckii*, are examples of bacteria that can be utilized in fermentation.



Figure 2.5: *Lactobacillus casei*

Microorganisms	Metabolite	Location within The Fermentation	Biological Activity	Downstream
<i>Lactobacillus lactis</i>	2,3 butanediol	Supernatant	Bulk chemical in plastic industry	Distillation, stream stripping, pervaporation
<i>Lactobacillus sanfrancisco CBI</i>	Caproic acid	Supernatant	Antimicrobial, Flavor and fuel precursor	Organic extraction
<i>Lactobacillus acidophilus</i>	Exopoly - saccharides	Supernatant	Antioxidant, antibacterial, antiulcer, antitumor, immunostimulatory	Ethanol precipitation
<i>Lactobacillus rhamnosus GG</i>	Lipoteichoic acid	Supernatant	Immunomodulator	Organic extraction, hydrophobic interaction, chromatography
<i>Lactobacillus reuteri</i>	Reuterin	Supernatant	Antimicrobial	Alcoholic extraction, organic extraction, size filtration and ion exchange, distillation

Table 2.4: Secondary metabolites produced by LAB compatible with the multi – product bioprocess (Jise Anibal Mora - Villalobos, 2020)

Furthermore, glycerol, format, pyruvate, succinate and mannitol were also examples of further by – products. Only homo-fermentative organisms have commercial value in the production of lactic acid, which produce optimum at temperatures between 37°C and 38°C at pH of 5 – 7. Several species have been identified that can produce largely isomer, as shown in Table 2.5.

L (+) lactic acid producer	D (-) lactic acid producer	DL – lactic acid
L. rhamnosus	L. coryniformis	L. acidophilus
L. amylophilus	L. bulgaricus	L. helveticus
L. bavaricus	L. jensenii	
L. casei	L. lactis	
L. maltaromicus		
L. delbrueckii		

Table 2.5: Lactic acid isomer produced by *Lactobacillus* species

2.13.1 Fermentation Operating Condition and Parameter

Lactic acid fermentation has been studied since 1035 using different types of microorganisms and fermentation operating factors such as pH, carbon sources, temperature, inoculum size, initial substrate condition and nitrogen source have also been investigated. A batch process in which nutrient absorption, cell multiplication and product collection, amongst with many others. Normally, carry out lactic acid fermentation. In addition, strains that grow well and produce large amounts of acidity on one carbon source might not be doing so on another. Furthermore, a few parameters and operating conditions effluence the optimal production of lactic acid generations. According to (Saprativ P.Das, 2017) stated that, temperature, pH and fermentative microbial inoculum volume are most needed in process parameter in lactic acid production.

2.13.2 Microbial strain

The selection of the production strain which is one the most important parameters for produce effectively production of lactic acid. Besides, in lactic acid production, strain development is focused not only at high yields and productivity, but also at the ability to reduced raw materials and to use substrates with chemicals that may be adversed to production conditions. Moreover, the selection of strains for these difficult properties has generally been observe and experimentation. Lactic acid can be produced by a large number of bacteria. The fermentation of various sugars is compared with *Lactobacillus* strains. The strains with the highest lactic acid concentration and yield also usually showed the highest productivity. On lactose, including whey and milk, *Str. thermophilus* was in most studies superior to *Lactobacillus delbrueckii spp. bulgaricus* and *L. lactis.. delbrueckii and lactic casei* shows the highest lactic acid concentration and yield production. According to (Seung Hee Jung, 2021) , *lactobacillus casei* had significantly larger rates in simulated gastrointestinal digestion over long – term fermentation than other types lactobacillus. In addition, lactobacillus casei can greatly enhance gene producing output tight junction protein during fermentation (Seung Hee Jung, 2021).

2.13.3 Effect of temperature

Temperature is one of the most important parameters that effects lactic acid production. Furthermore, some researchers have looked into the effect of temperature on lactic acid production and determined the optimum temperature between 41°C – 45°C. Lactic acid bacteria main disadvantage is that they needed higher complex (Elahe Abedi, Seyed Mohammad Bagher Hashemi, 2020) nutrients and lower fermentation temperatures which is (45°C) than other microorganisms, due to the higher costs, higher risk of contamination and a weakening of the products. Plus, lactobacillus casei is mesophilic bacteria that grows at temperature of 17 °C to 50 °C, with the optimal temperature range between 20 °C to 50 °C. Besides, the yield increases same with increases in level of fermentation temperature at 30 °C to 50 °C.

2.13.4 Effect on pH

There are different methods to control the pH of the fermentation process. It can be established at the beginning and then effectively decreased due to acid fermentation. Even more, when the pH is adjusted, basic titration can be carried out. The pH fermentation is controlled by basic titration, lactic extraction, adsorption or electro-dialysis acid or it is controlled at the start and then reduce due to acid production. In addition, several researchers have looked into the effects of pH on lactic acid production and identified that optimal pH for lactic acid production is between 5 – 7. According to (Whiny Erliana, 2020), *Lactobacillus casei* and *Lactobacillus rhamnosus* were used in a field lactic acid fermentation process with different value of pH (4.5,6.5,7.5) and temperature (29°C, 33°C,37°C, 41°C). Moreover, after 48 hours of incubation, the largest lactic acid concentration was identified. *Lactobacillus casei* produced 4.261 g/L and *Lactobacillus rhamnosus* produced 3.523 g/L at 37°C with a pH of 6.5. The concentration and yield of lactic acid were decreased to 21.88 g/L and 31.25% respectively when the fixed pH has been raised to 6.5 (Sumit Kumar, 2020). Furthermore, the lower production rate of 11.59 g/L or 16.55% yield was resulted with the lowest pH of 5.5. In all conditions, titration to a constant pH results in lactic acid concentrations, yields and productivity which are higher or equal to obtain with no pH control.

2.14 Fermentation mode

Lactic acid is often produced in a group environment, but however there are multiples examples of continuous culture, as well as several feeding and semi – continuous groups/repeated group fermentation. In most studies, when comparing groups and continuous fermentation mode, the former produces higher lactic acid concentrations and yields. This is primarily due to the facts that all substrates are employed in cluster mode and the residual concentration remains constant.

In contrast, continuous mode usually yields higher productivity. The main reason may be a continuous culture of running high dilution rate, where the advantage over cluster mode is most visible. In continuous culture, adjusting the dilution rate has such an impact on both substrates and nutrient concentrations. However, the impact on yield and productivity is not yet complete. In this section, the type of bacteria selected, as well as the range of operating parameters, will be briefly described in this part to provide framework for this study and to support in the selecting of appropriate microorganisms and operating setups for lactic acid fermentation of food waste.

2.15 Continuous fermentation

The basic fermentation process mostly is batch. The culture is grown in a series of inoculum tunnels until being transferred to the production fermenter. Furthermore, the inoculum size is normally 5 – 10% of the volume of liquid in the fermentor. Fermentation is normally controlled at 35 - 45°C and pH 5 – 7 with addition of effectively utilized such as ammonium hydroxide. Eventually, lactic acid fermentation was achieved using lactic acid bacteria at 37°C and pH 6 for 48 hours (Whiny Hardiyati Erliana, 2020).

Furthermore, the highest lactic acid concentration (33.292 g/L) was achieved using mixed culture *Lactobacillus casei* and *Lactobacillus brevis* to convert reducing sugars into lactic acid (Whiny Hardiyati Erliana, 2020). Product concentration of lactic acid has been reported as 61 g/L and 94% respectively (Bayraktar, 2015). According to (Andrea Komesu J. A., 2017), lactose fermentation with *L. casei* yield optically pure D(-) lactic acid. Both the presence of lactic acid in fermentation and the subsequent pH reduction, that lowers the ability of cells to produce lactic acid are the major limitations of the batch fermentation process. Adding a basic solution such as Ca-lactate precipitates and inhibit pH decrease. However, this precipitate should be dissolved with different acids, such as sulfuric acid. Although this process is not technically complicated, it is pricy on a large scale and required a large amount of additional chemicals.

2.16 Substrate of lactic acid production via fermentation

Lactic acid has been widely produced using several carbohydrate substrates through fermentation. Since 1950, sucrose has refined from sugar cane and beet sugar also is the most widely used substrate, followed by dextrose and maltose from hydrolyzed starch (Cristian Torres Leon, 2018). Furthermore, sugar and starch also have quality of food and its resources is limited. Some raw materials or by – products have been studied as a potential low – cost lactic acid substrate.

In addition, the raw elements for the fermentation process contain of carbohydrates and nutrient for cell growth. Moreover, lactose from whey or hydrolyzed corn syrup are the most common carbohydrates used in large – scale fermentation. The latter is basically glucose, with some higher saccharides thrown in the mix. For the production of lactic acid through fermentation, a variety of carbohydrates materials have been applied, tested or recommended. Table 2.6, summarizes the substrates for lactic acid fermentation.

Principle substrate	Source
Lactose	Casein whey Cheese whey Sweet whey
Glucose	Corn
Sucrose	Molasses Cane sugar Beet sugar
Other	Potatoes Cellulose Sorghum extract

Table 2.6: Summary of the substrate for lactic acid fermentation ((Bintsis, 2018)

It is useful to compare raw materials based on the qualities that prefer:

- Low cost
- Low levels of contaminants
- Fast fermentation rate
- High lactic acid yield
- Ability to be fermented with little or no pretreatment

Raw materials have been avoided due to the high level of external materials, the material can cause segregation problems in the recovery stage. Use of pentose sugar results in the production of acetic acid which will bear the additional process equipment for separating. The results suggest that whey cheese is a good, low – cost substrate for lactic acid production. However, there is still a fixed number of whey obtainable.

2.17 Lactic acid producing microorganisms

Lactic acid is an organic compound produced through fermentation process by using different microorganisms such as different carbohydrate sources. Furthermore, lactic acid bacteria are the common bacteria that used to produce lactic acid and lactobacillus are the one of bacteria showing interesting into lactic acid (Elahe Abedi S. M., 2020). Uses of *Bacillus* spp. showed a good probability to turn down the cost of fermentation process. Amazingly, strong productivity of lactic acid can be perform with *Corynebacterium glutamicum* and *E. coli*, primarily after designed genetic modification (Elahe Abedi S. M., 2020).

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, well known as isomer produced by selected lactic acid which producing bacteria and fungi. Fermentation with produce the microorganisms and their yield of lactic acid production. Hetero fermentative LAB produce 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.

2.18 Types of microorganisms

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 microorganisms metabolism differs with different sources (Andrea Komesu J. A., 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 S. M., 2020). This compound can be synthesized via the hexoses and pentose lactic acid bacteria (LAB) metabolism pathways as shown in Figure 4. 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.

2.19 Autoclaving

Autoclaving is the one of efficient method of sterilizing. Specific for laboratory equipment, liquid handling product to kill harmful bacteria, viruses, fungi and spores. The autoclaving methods take advantage on the phenomenon on the boiling point of water (or steam), increases when it is under high pressure. It is performed in a machine known as an Autoclave where uses high pressure recommended at temperature 250 °F (121 °C) for 15 – 20 minutes to sterilize the equipment. Autoclave or steam sterilization are used in several industries such as medicine, dentistry, microbiology and veterinary science (Jean Fulbert Ituna Yudonago, 2021).

In current era of globalization, medical and laboratory settings both are using autoclave to sterilize the laboratory equipment and waste. Autoclave sterilization work by using heat to kill the microorganisms including bacteria and spores. Moreover, the heat moved through pressurized steam. The pressure enables the steam to reach the high temperature required for sterilization. There have a few methods of sterilization of medical equipment checking including steam sterilization, chemical sterilization, dry heat sterilization, plasma gas sterilizer, vapour hydrogen peroxide sterilization and plastic tool sterilization basket (Jean Fulbert Ituna Yudonago, 2021).

According to the Centers for Disease Control (CDC), Guideline for Disinfection and Sterilization of Healthcare Facilities, pressure steam is the most regularly and reliable method of sterilization. It is non – toxic and low cost, kills bacteria, spore speedily heat up and penetrates fabrics (C. Netzel, 2021).

2.19.1 Autoclaving Process

Autoclave is the chamber that sterilize a medical or laboratory instrument by heating it out over the boiling point. Moreover, most clinics have a table – top autoclave, well about the same size of a microwave. Mostly, hospital and clinic are using the large autoclave, also called horizontal autoclave. Currently, autoclave is widely used in hospitals and health organisations as sterilization of medical equipment (Jean Fulbert Ituna Yudonago, 2021).

The autoclave process is started with locked the autoclave door using chamber closed. According this time, the vacuum pump removes all the air present in the chamber and replaces it with steam. Thus, pressure is applied to the steam to achieve the desired period of time. Once the cycle is complete, steam is depleted and laboratory equipment is carefully removed from the chamber. Related to autoclave process, there have 3 phase of sterilization cycle, which is Purge Phase, Exposure (Sterilization) Phase and Exhaust Phase. The autoclaving process is not practical for many materials because of high temperature involved. Autoclave is compatible for steam sterilization at the recommended temperature.

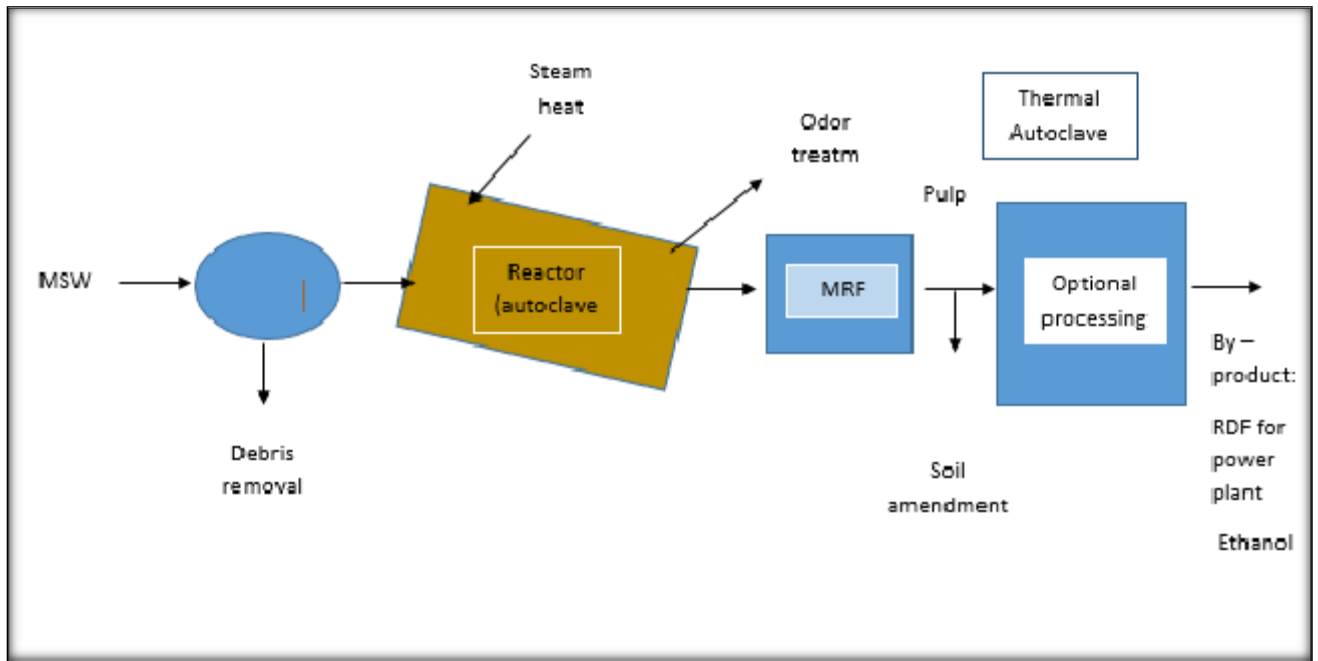


Figure 2.6: A basic Autoclave process ((Rogoff, 2018)

2.19.2 Autoclaving procedure

Autoclave uses the pressurized steam to remove microorganisms, which is the most attested system for decontamination of laboratory waste and sterilization of laboratory glassware, media and reagents. According to (L. Angel, 2016), an autoclave is a metal equipment with sealing chamber which is used to conduct the sterilization process using high - pressure steam. For the best result to efficient heat transfer, the steam must turn off from the autoclave chamber. Before start using the autoclave, check the drain screen at the bottom of the chamber and clean if its blocked. If the filter blocked with debris, a layer of air might form at the bottom of autoclave and effecting efficient operation.

Container selection for autoclave method which is polypropylene bags, polypropylene containers and pans, and stainless steel containers and pans. Usually, it called as biohazard or autoclave bags. These bags are tear resistant but can be puncture or burst in an autoclave. Moreover, another procedure which is preparation and loading materials, cycle selection, time selection and removing the load. Especially for medical sterilization autoclave, which is the process including Pre – Vacuum, Rising stage

Temperature, Sterilization and Vacuum Drying, where it has different dynamic properties (L. Angel, 2016).

2.19.3 Autoclaving in term of food waste

Waste autoclaving is a kind of physical solid waste treatment in uses processed using the heat, steam and pressure of an industrial autoclave. In autoclave process, wastes are collected in batches or in a continuous flow process. According to batch process, saturated steam is poured into an autoclave at a temperature around 160 °C. The autoclave system is operation mechanical treatment technique, that involves saturated steam under high pressure to treat mixed industrial waste and municipal solid waste (MSW). Furthermore, autoclaving is combination with mechanical isolation as well as mechanical heat treatment. Recently, the autoclave has recently been included in the treatment of municipal solid waste compared to other solid waste treatment process, which is use autoclaves in solid waste treatment ongoing bairn (Nurazim Ibrahim, 2018).

Food waste (FW) autoclaving for resource recovery and reuse was performed by producing lactic acid production. The experiment was conducted in stated temperature in a few hours. In addition, autoclave pre-treatment operation into sterilize waste and removes infectious substance and odors, thus allowing the waste to be recycled more efficiently (Chia Chi Chang, 2018). Furthermore, the bio – waste autoclave can simply produce solid organic flocc/fiber without compiling solid products with little inorganic content.

CHAPTER 3

METHODOLOGY

Introduction

Based on previous studies, the optimal condition for fermentation process was achieved at pH 6.5 - 7, temperature 37°C and inoculum size of 4% (v/v) with a 120 h incubation time and 150 –rpm effective rotation speed (Seung Hee Jung, 2021). In this initial research studies on lactic acid fermentation using immobilized *lactobacillus casei*, and *lactobacillus acidophilus* research comprised of several phases. The first phase of this study compares the differences between multiple food waste that using microorganisms to produce lactic acid production. For the final stage, food processing waste from bread waste, fruit waste and domestic waste as a raw material has been used for treatment process (fermentation). In this section explains more about the research framework. Materials, types of food waste and analytical method has been demonstrated clearly. The details of the experiment outcome have been discussed further in the relevant chapters. Figure 3.1 elaborates the research framework to provide crystal – clear view on the experimental strategy.

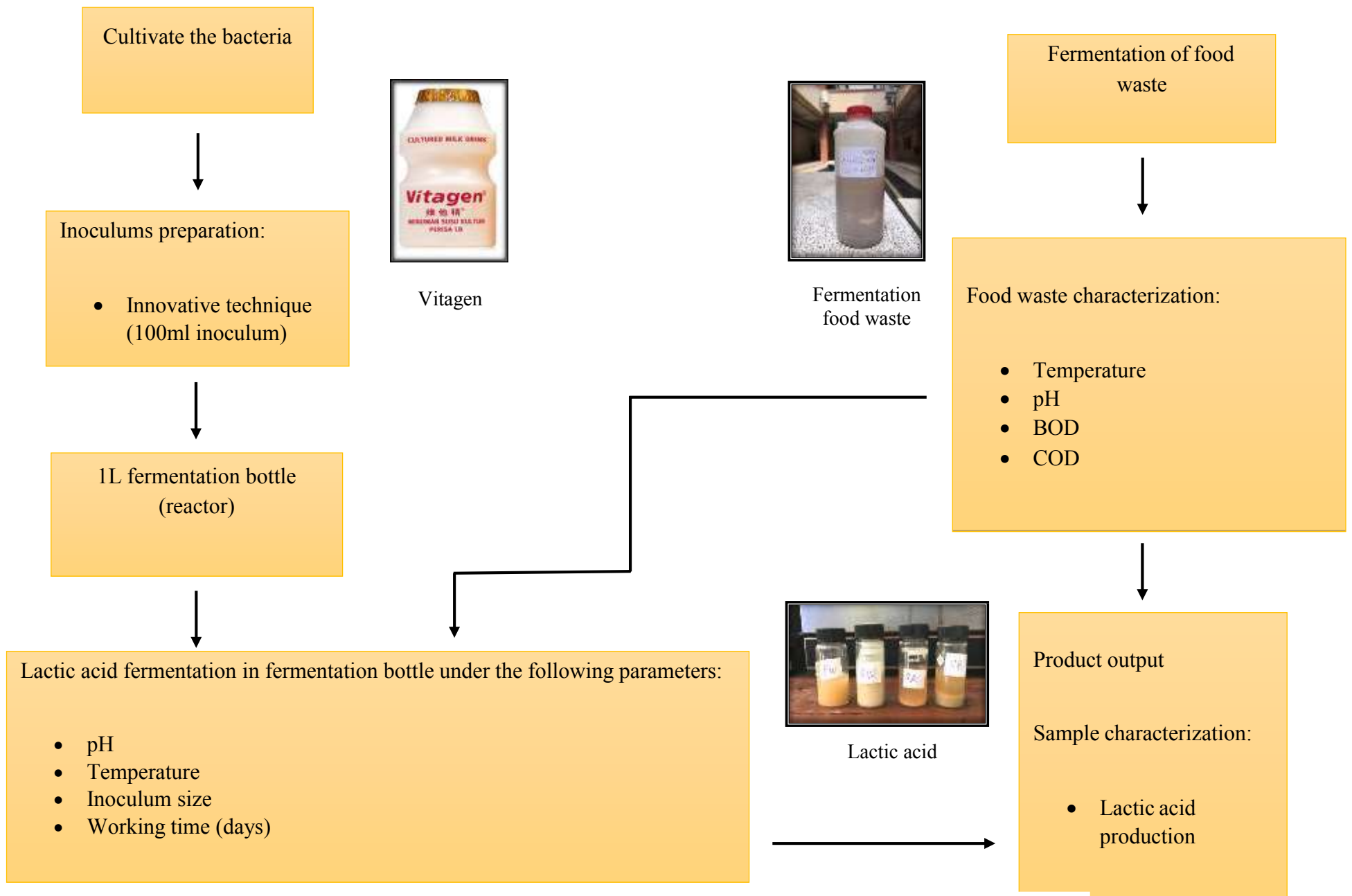


Figure 3.1: Schematic diagram summarizing the experimental methodology

3.1 Material and Methods

3.2.1 Chemicals

Basically, the chemicals needed for this experiments in this studies were divided into two categories which is chemicals for fermentation of food waste residues (medium) and chemicals for HPLC observation using mobile phases. The chemical used are analytical grade and purchased only from one suppliers. The lactic acid standard used in this study was obtained from food waste standard.

3.2.2 Strain

The microorganisms used in this experiment was live probiotic cultures *Lactobacillus acidophilus* and *Lactobacillus casei* from vitagen. In addition, *lactobacillus casei* is a mesophilic homo - fermentative lactic acid bacteria (Bintsis, 2018). It comes from industrial market and easy to find it.

3.2.3 Food Waste Source

The food waste used throughout the experiment was collected from university cafeteria (University Malaysia Pahang). They contain commonly of leftover food, fruit and bread. The water content of the food waste is around 80% (w/w). Later, the wet food balance samples were applied as substrate directly. The wastes stored at -4 °C deep freezer pending the fermentation and analysis.

3.2 Experimental Methods

3.3.1 Preparation of Food Waste

A food blender was used to grind 1L of water with 500g of food waste for 5 minutes, and the process was repeated for four different samples. Then, the samples were stored in the chiller until the experiment started.

3.3.2 Inoculum Preparation

Lactobacillus acidophilus and *Lactobacillus casei* (live probiotic cultures) were found in culture milk, Vitagen. The probiotic culture was transferred to a 100ml beaker. After that, the inoculum was transferred into the sample after the autoclave process. The growing colonies are either used to make inoculum or stored at 4°C for later use. 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 reduce product contamination (Bintsis, 2018).

3.3.3 Autoclave

According to this experiment, 500ml of food waste were transferred to the 1-liter fermentation bottle before being put in HICLAVE (HVE – 50) culture vessel for sterilization process. The temperature was maintained at 150°C and the time at 15 minutes by using saturated steam under at least 70 kPa of pressure. Check the vessel and make sure the vessel is empty and free from any possible inhibitor. Put the food waste that needs 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. The HICLAVE autoclave machine is set-up as shown in Figure 3.2.



Figure 3.2: Autoclave set - up

Safety valves are adjusted to keep the necessary pressure inside the vessel. Once the water starts to be boiled up, the vapor is allowed 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 70 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.



Figure 3.3: The sample after sterilization

3.3 Fermentation of food waste

The potential of indigenous bacteria to convert substrates into lactic acid can be determined by analyzing the level of lactic acid accumulated in fermented food waste media. Furthermore, food waste is homogenized to a semi – solid consistency and fermented in this section.

3.4.1 Chemical composition of food waste

The food waste (FW) of 500g is grounded with 1L water in a food blender until all fine. About 100ml of homogenized food waste uses in chemical analysis. Lactic acid production was determined by using this method:

3.4 High – performance liquid chromatography

Lactic acid can be determined using high – performance liquid chromatography. This standard was constructed using one concentration of lactic acid prepared from concentrated liquid lactic acid 1 mole and 5 ml double distilled water. The standard was prepared for 1 mole to 5 ml of double distilled water. According to this research, double distilled water is very important to remove any interference of metal ions contained in ordinary tap water and distilled water which can effect color formation. All preparation has setup using high – performance liquid chromatography (HPLC).

3.5 Methodology flowchart



3.6 Analytical Methods

3.6.1 pH

The accurate and practical technique for measuring pH require the use of pH meter. The pH meter is a potentiometer that determine a developed potential between the glass electrode and the reference electrode. To get the accurate results, the pH meter should be calibrated before use. Furthermore, the calibration usually performed using a standard pH meter with standard pH buffers of 4.00, 7.00 and 9.00. Moreover, when using a pH meter, make sure the electrode is completely saturated with the test solution and soaked to a sufficient depth. The pH reading will be recorded after a minimum five minutes.

3.6.2 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 dissolve 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.

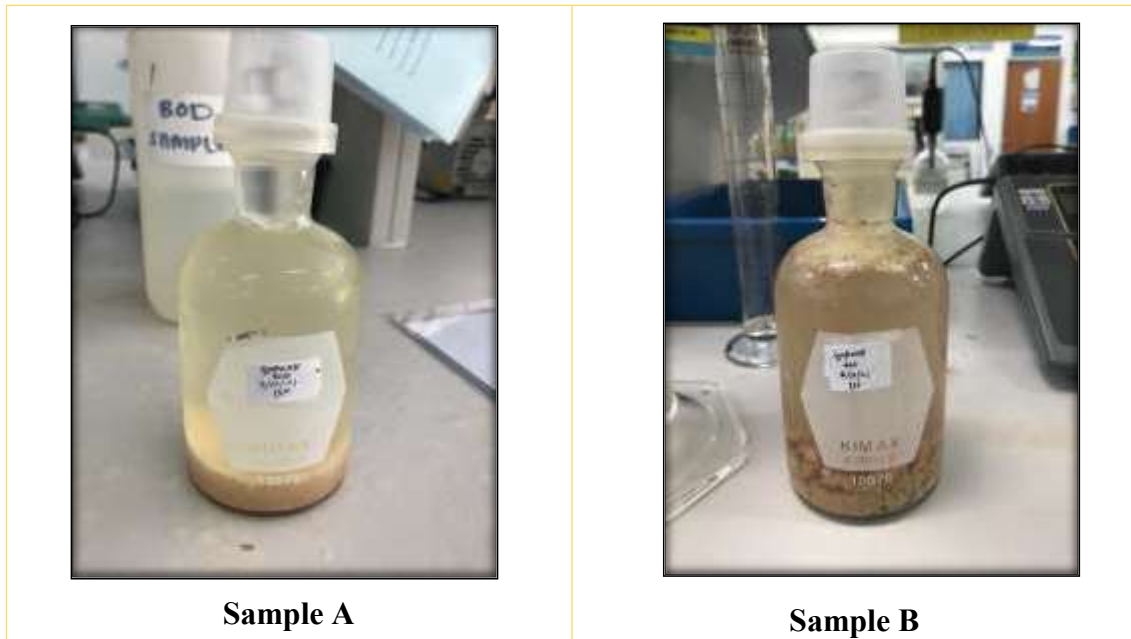


Figure 3.4: The BOD result for sample A and B after 5 days

3.6.3 COD

COD digestion reactor were set up in 150° 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 °C 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.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Introduction

This chapter will show the according to the objectives in this study. The validation test on treatment process of food waste and develop lactic acid production from food waste using fermentation process has been completely performed in this study. The experiment could be run for the part of analysis before and after treatment process and determination of other product lactic acid that can be produced after fermentation was achieved. The analysis process is to determine the effectiveness of food waste treatment in order to produce the product after the treatment and also to minimize the production of food waste in daily life.

According to this research, experimental results were obtained for food waste treatment and lactic acid production with respect to the parameter has been measured which pH, BOD and COD and the percentage of efficiency of food waste treatment using the varies food waste sources.

4.2 Fermentation Process

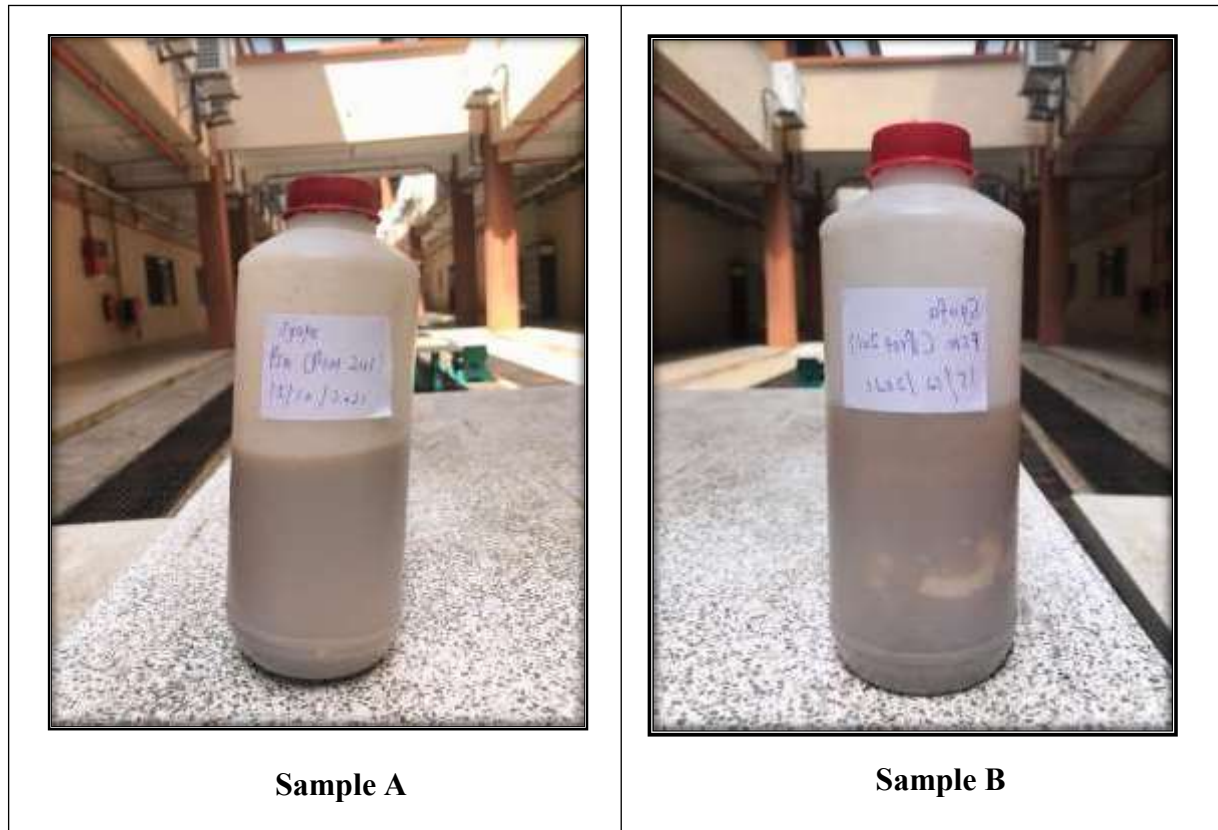


Figure 4.1: Reactor of fermentation process

Figure 4.1 showed the reactor and finished fermentation process after grinded food waste and additional inoculum into sample. All the food waste sample were in good conditions to be tested and observed. Also all the parameter for food waste standard has been determine before and after fermentation process during 15 days.

The fermentation process for all samples were carried out at hostel and environmental laboratory due to the COVID – 19 pandemic happen which cause the limitation movement to perform the experiment. The processes finished were on time and followed within the allocated time. Raw materials for this project were collected at the cafeteria University Malaysia Pahang, with different types of food waste.

The parameter test was done for all samples to determine the growing bacteria in food waste before and after treatment process. Based on the observation, the most efficient food waste that shows the consistence parameter and growing bacteria was domestic waste. So, compared to the other sample, the efficiency and high yield production of food waste treatment are not shows the great results. Furthermore, according to results shows that domestic waste can produce the higher yield of treatment process compared to agriculture waste. Plus, in process to observe lactic acid from food waste, the result shows that only domestic waste has lactic acid production. For agriculture waste does show any result that has lactic acid.

Overall process of the fermentation process working properly after some modification made especially on the microorganisms that used to culture the food waste during the treatment process. The pH reading before and after treatment shows the decreasing value which is the good condition on lactic acid production.

4.3 Performance Analysis

4.3.1 BOD different before and after fermentation process

Sample	Temperature (°C)	pH	BOD (mg/L)
A (Agriculture waste)	28	6.72	388
B (Domestic waste)	28	6.56	350

Table 4.1: BOD result at 28 °C before fermentation process

Sample	Temperature (°C)	pH	BOD (mg/L)
A (Agriculture waste)	28	4.66	135
B (Domestic waste)	28	4.32	108

Table 4.2: BOD result at 28 °C after fermentation process

4.3.2 COD different before and after fermentation process

Sample	Temperature (°C)	pH	COD (mg/L)
A (Agriculture waste)	28	6.72	11488
B (Domestic waste)	28	6.56	6912

Table 4.3: COD result at 28 °C before fermentation process

Sample	Temperature (°C)	pH	COD (mg/L)
A (Agriculture waste)	28	4.66	568
B (Domestic waste)	28	4.32	432

Table 4.4: COD result at 28 °C after fermentation process

4.4 Effectiveness (%)

4.4.1 Percentage (%) of BOD at 28°C before and after fermentation process

Sample	(%) BOD	(%) BOD
	before	after
A (Agriculture waste)	53	47
B (Domestic waste)	56	44

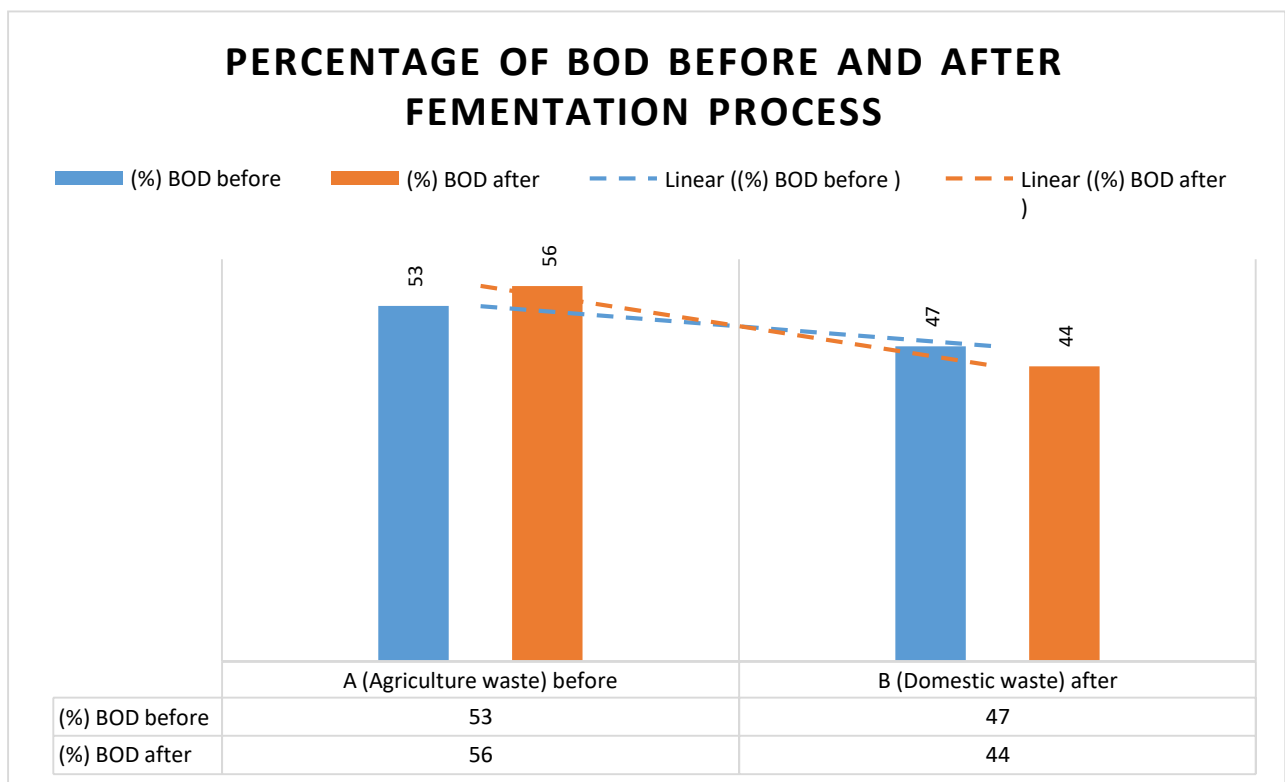


Figure 4.2: Percentage (%) of BOD at 28°C before and after fermentation process

4.4.1 Percentage (%) of COD at 28°C before and after fermentation process

Sample	(%) COD	(%) COD
	before	after
A (Agriculture waste)	62	38
B (Domestic waste)	50	39

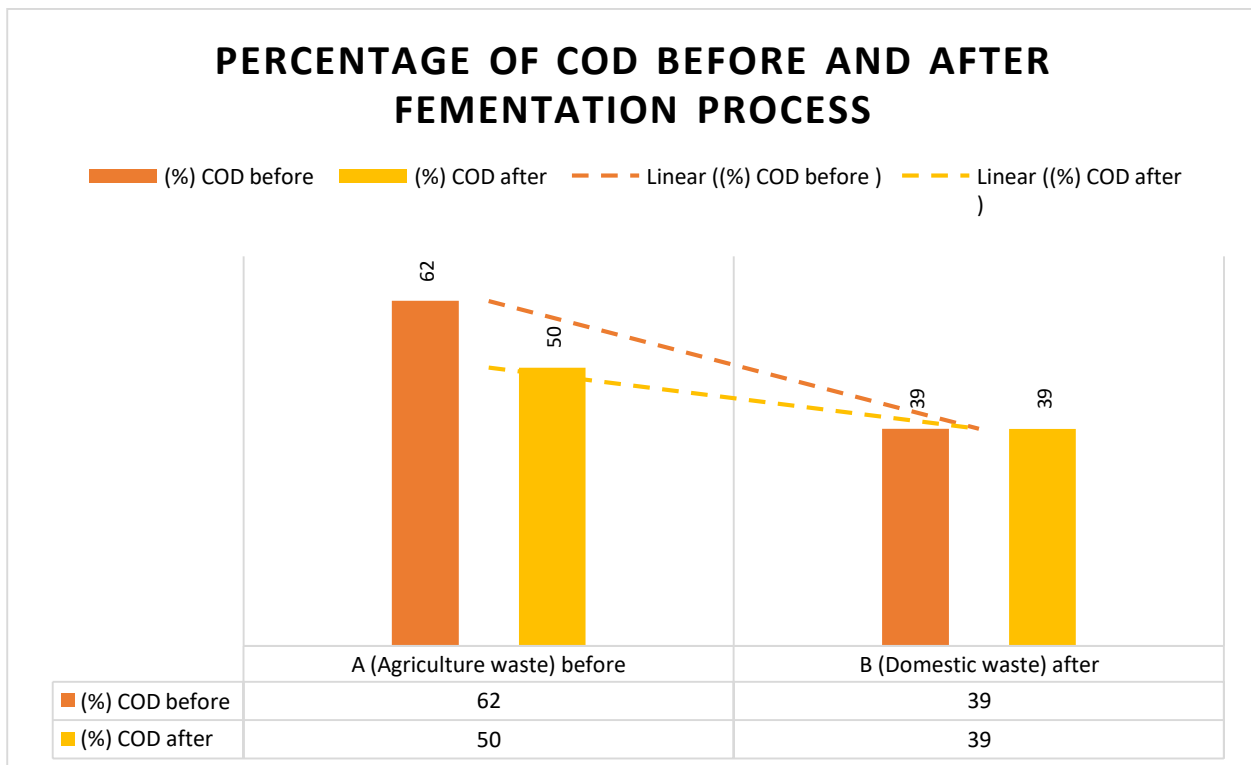


Figure 4.3: Percentage (%) of COD at 28°C before and after fermentation process

According to Figure 4.2, indicates the percentage of BOD readings for agriculture waste is decreased from 53% to 47%. BOD test can be measured by using an approximate dilution range with the sample volume in the BOD bottle. This condition happened because of the rate of oxygen consumption is affected by the number of variables that has been measured in this experiment including, temperature, pH, and the existence of various microorganisms inside the food waste. In this experiment, pH value for agriculture waste before treatment has been stated at 6.72 and after treatment is 4.66, and room temperature at 28°C. From the result, the percentage of decreasing is between 6% before and after fermentation process which shows that the lower BOD value are also influenced based on the food waste that has been used as a sample. Next, the percentages reading for domestic waste is also shows the result decreased from 56% to 44% which the value of decreasing is 12%. This value is higher that agriculture waste before and shows that domestic waste is more effectiveness after the treatment process happened. The pH value and temperature for domestic waste has been stated at 6.56 before treatment and 4.32 after treatment, and room temperature at 28°C.

In addition, Figure 4.3 shows the percentage of COD readings for agriculture waste which is decreased from 62% to 39% and domestic waste is 50% to 39%. Basically, COD test is usually used as indirectly to calculate the amount of organic compound in the water. Based on result, it shows that both sample from agriculture waste and domestic waste reading are decrease around 23% and 11%. This situation happened because of the increasing time as microorganisms in the water will grow and digest the organic substances in the samples. The pH and temperature was controlled and same with BOD test before.

The value of overall BOD and COD is decreasing because of the few parameters that already set for both sample which agriculture waste and domestic waste. Overall BOD and COD reading for both sample is decreased which is suitable condition to perform the treatment process. Besides if COD content level high in waters, will caused ecological disruption, deterioration of water quality and reduction in water self – purification capability (Netti Herlina, 2019).

4.5 Lactic acid stock solution calculation

The formula used to determine the volume of stock solution be used is:

Normality Formula:

$$N_1V_1 = N_2V_2$$

Where:

N_1 = Assumed amount of stock solution

V_1 = Unknown value of double distilled water (x)

N_2 = Volume of stock solution

V_2 = Volume of double distilled water

i. For 1mole solution, (Double distilled water, 5ml)

$$N_1V_1 = N_2V_2$$

$$90 \text{ M} \times (\text{xml}) = 1 \text{ M} \times 5\text{ml}$$

$$(\text{xml}) = \frac{5 \text{ ml} \times 1 \text{ m}}{90 \text{ m}}$$

= 0.05 ml of stock solution mixed with 5ml of double distilled water.

ii. For 2mole solution, (Double distilled water, 5ml)

$$N_1V_1 = N_2V_2$$

$$90 \text{ M} \times (\text{xml}) = 2 \text{ M} \times 5\text{ml}$$

$$(\text{xml}) = \frac{5 \text{ ml} \times 2 \text{ m}}{90 \text{ m}}$$

= 0.11 ml of stock solution mixed with 5ml of double distilled water.

iii. **For 3mole solution, (Double distilled water, 5ml)**

$$N_1V_1 = N_2V_2$$

$$90 \text{ M} \times (\text{xml}) = 3 \text{ M} \times 5\text{ml}$$

$$(\text{xml}) = \frac{5 \text{ ml} \times 3 \text{ m}}{90 \text{ m}}$$

= 0.16 ml of stock solution mixed with 5ml of double distilled water.

iv. **For 4mole solution, (Double distilled water, 5ml)**

$$N_1V_1 = N_2V_2$$

$$90 \text{ M} \times (\text{xml}) = 4 \text{ M} \times 5\text{ml}$$

$$(\text{xml}) = \frac{5 \text{ ml} \times 4 \text{ m}}{90 \text{ m}}$$

= 0.22 ml of stock solution mixed with 5ml of double distilled water.

v. **For 5mole solution, (Double distilled water, 5ml)**

$$N_1V_1 = N_2V_2$$

$$90 \text{ M} \times (\text{xml}) = 5 \text{ M} \times 5\text{ml}$$

$$(\text{xml}) = \frac{5 \text{ ml} \times 5 \text{ m}}{90 \text{ m}}$$

= 0.28 ml of stock solution mixed with 5ml of double distilled water.

4.6 Lactic acid result using HPLC

4.6.1 Standard Lactic Acid 5mol

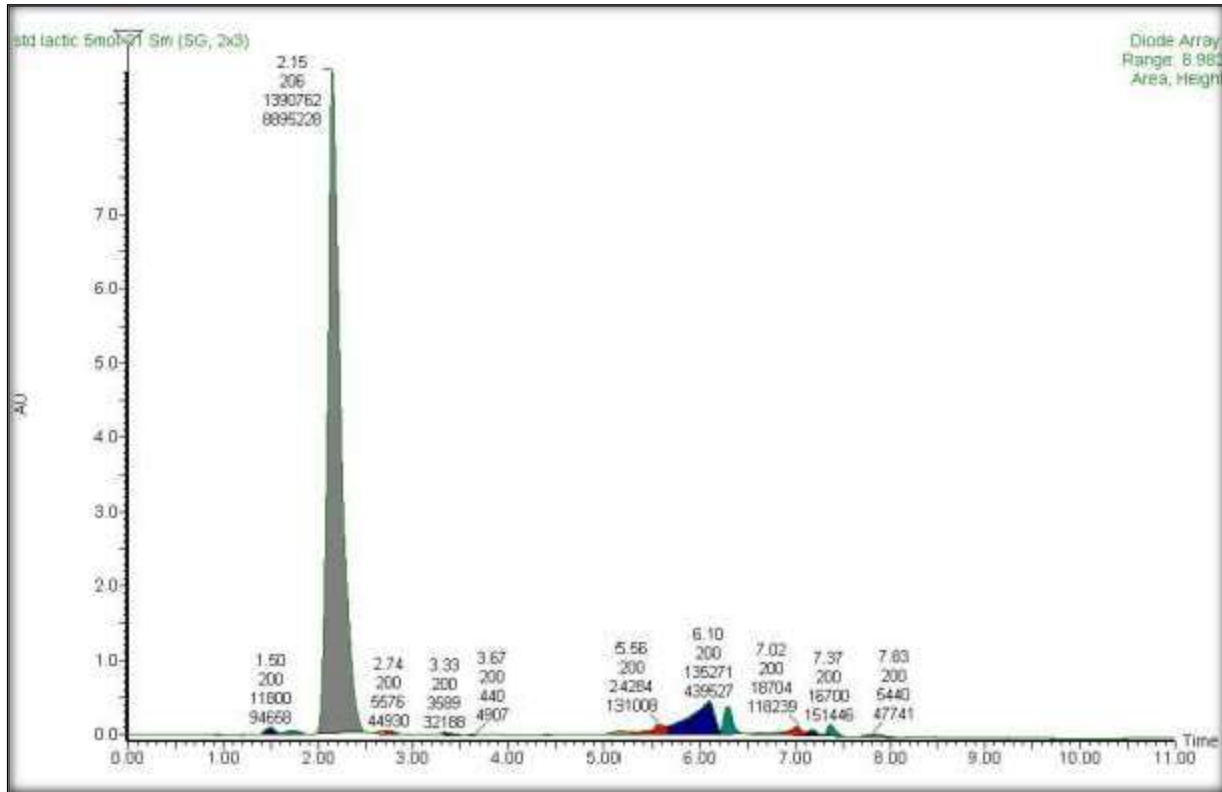


Figure 4.4: Determination of lactic acid in standard time vs standard

COMPOUND NAME	RET. TIME (min)	AREA	HEIGHT
Lactic acid	2.15	1390762	8895228

Table 4.5: Lactic acid standard

4.6.2 Lactic acid observation in sample A

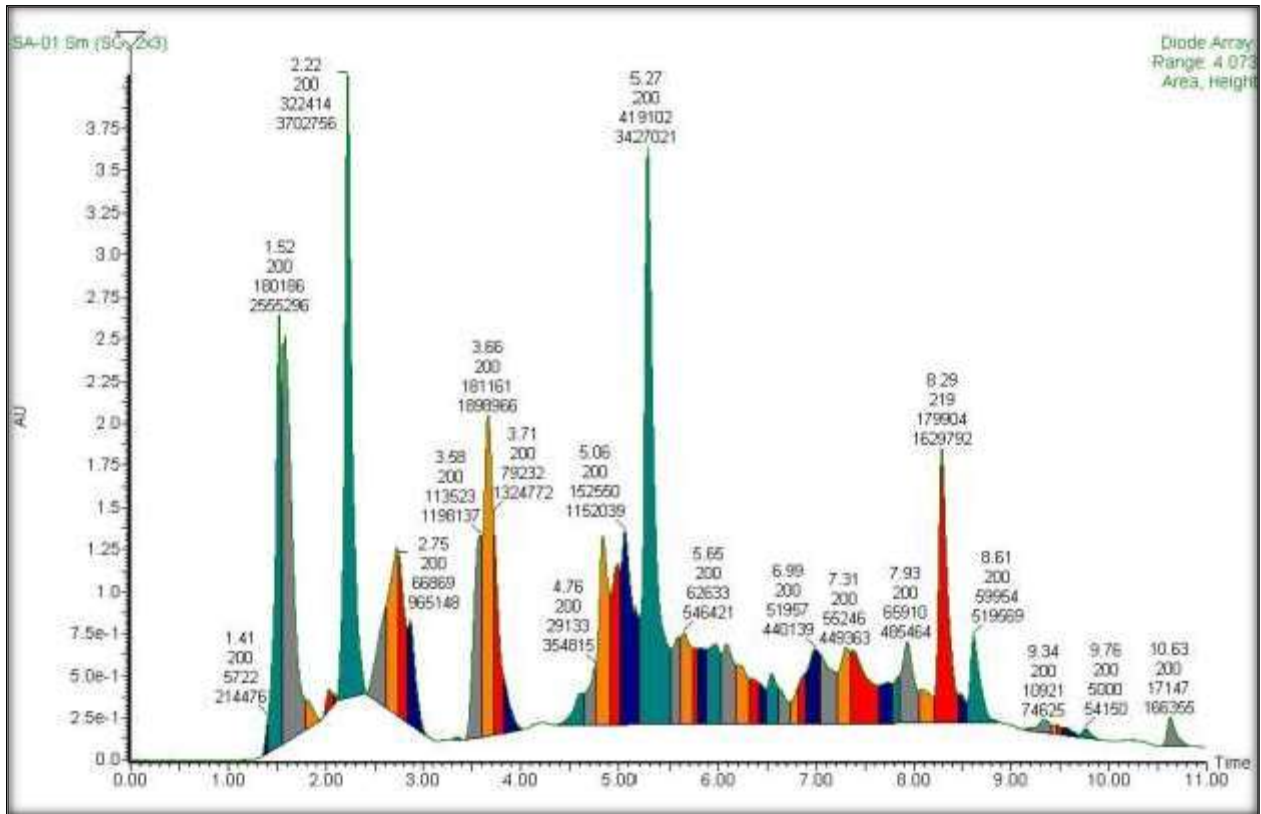


Figure 4.5: Determination of lactic acid in sample A

COMPOUND NAME	RET. TIME (min)	AREA	HEIGHT
No result for Lactic acid	-	-	-

Table 4.6: Lactic acid value in sample A

4.6.3 Lactic acid observation in sample B

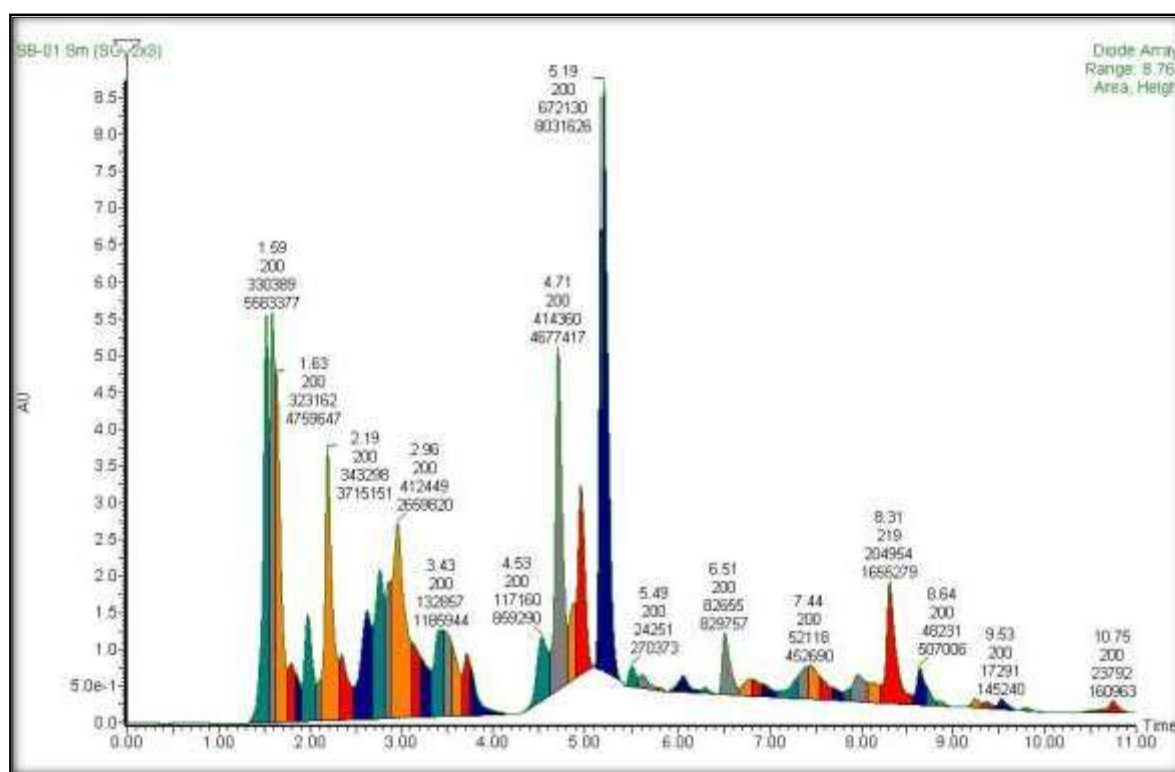


Figure 4.6: Determination of lactic acid in sample B

COMPOUND NAME	RET. TIME (min)	AREA	HEIGHT
Lactic acid	2.19	343298	3715151

Table 4.7: Lactic acid value in sample B

From Table 4.5 shows that the result of sample A which is from industrial waste. According to observation using High Performance Liquid Chromatography (HPLC), there is no lactic acid can be produce or perform from agriculture waste. This situation happen because of the composition of microorganisms inside the food waste after the fermentation process is die which is the microorganisms not growing after additional inoculum afterwards autoclave process. That has proven that agriculture waste can be reused using treatment process but can't produce value able product that can give benefit into human also increasing the industrial economic percentages of renewable product from waste materials.

In addition, from Table 4.6 shows that, the result from sample B which is from domestic waste. Relating to the result, its show that domestic waste can produce lactic acid after the treatment process. The percentage of the lactic acid that has been produce is in 57% which it is quit higher achievement and proving that domestic waste can be a renewable product that can be uses in future. Furthermore, have many recent work has focused on the production of lactic acid from a variety of food waste such as restaurant, university kitchen and agricultural waste (Ali Nawaz, 2017).

Overall from this research, it can be concluded that food waste production can be reduce using the alternative method such it is easy to perform and applied into the industrial which is treatment process (fermentation). Lactic acid production also can be produce in multiple method such as fermentation of sugar and food waste (Ashfaq Ahmad, 2021). Furthermore, can be applied in different processes which is simultaneous saccharafication, solid submerged fermentation and hydrolysis (Ali Nawaz, 2017). Plus, in this study only focusing in fermentation process because this process does need high cost to run the experiment and also does not required for used a lot of equipment.

CHAPTER 5

CONCLUSIONS AND FUTURE SUGGESTIONS

5.1 Conclusions

In conclusions, comparison between using the different sources of food waste shows that the efficiency of treatment process to food waste is as potential to be used in lactic acid production. The important key components such the microorganisms that used to culture the food waste, the suitable temperature, pH value, condition and also the parameter tested on food waste also important to give the attention. From the two sample that have been tested, only food waste namely domestic waste produces lactic acid yield. The parameter BOD comparison between two sample during before and after treatment shows that the higher value and for COD reading is lower after treatment process happened. This situation happen because of the water is has low content of organic matter and has low amount of microorganisms. According to COD parameter that produce higher reading because of yield oxygen – equivalent that has higher value than BOD₅ because more oxygen equivalent can oxidize by the chemical than can be oxidize by microorganisms.

The fermentation condition (duration, temperature and initial pH) for fermentation of food waste are 15 days, 28°C and pH 7 respectively. According to two samples that has been used in this study, only domestic waste produce lactic acid production at retention time 2.19 min compared to agriculture waste that can't detect any lactic acid in that sample. Furthermore, at low initial pH, no lactic acid can be produced but as initial pH becomes more alkaline, more lactic acid is produced. Highest lactic acid yield is obtained at initial pH 7. Adjustment of initial pH 7 in different samples of food waste shows suppressed amount of lactic acid.

Lactic acid yield in food waste is enhanced when the suitable microorganisms is added in ratios of 100 percent of vitagen and increased the efficiency of food waste treatment using fermentation process. lactic acid yield will be decreased in the condition of microorganisms inside the food waste can't grow continuously. About 60 -70 percent of efficiency food waste was achieved when 100 percent of inoculum was added to both food waste samples.

5.2 Recommendations for Future Studies

The production of food waste treatment using fermentation process is a very promising waste management strategy. In addition, this method has low cost which is easy to implement into industry sectors. Furthermore, this treatment process also can produce the value able product which is lactic acid. Lactic acid can be used in manufacture cleaning agents, transformed to technical grade lactic acid or purified to a high purity lactic acid that can be utilized to produced poly-lactic acid polymers (PLA). Moreover, domestic waste has the potential to be evolve into natural fiber source for the production of prebiotics as cellulose helps the growth of good bacteria such as lactic acid bacteria. The comprehensive on the fermentative capability of domestic waste can be further explored through microbial colonies evaluation of the waste and quantitative determination of lactic acid. Lactic acid could be improved by inclusion of lactic acid bacteria inoculums and pretreatment such ad fermentation of food waste to increased accumulated yield.

The fermented waste obtained after filtration contains most of the fiber. Plus, this waste can be used in composting to facilities the process or it can also be used as soil amendments to help maintain soil structure and nutrient content.

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Appendix A

FERMENTATION PROCESS OF FOOD WASTE



Appendix B

LACTIC ACID SOLUTION PREPARATION



ACTIVITIES	WEEK													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Group member meeting	█													
Title briefing from Supervisor		█	█											
Discussion on project title			█	█	█	█	█							
Literature survey				█	█	█	█							
Analyze method experiment					█	█	█	█						
Finalize the method					█	█	█	█						
Material estimations						█	█	█	█					
Proposal first draft preparation							█	█	█	█				
Preparation slide presentation									█	█	█			
Finalize the proposal and submission final draft										█	█	█	█	
Material Purchasing											█	█	█	█

Appendix C: GANTT CHART SDP 1

ACTIVITIES	WEEK															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
SDP 11 Briefing	█															
Material Purchasing		█	█	█	█											
Collection of sample				█	█	█	█	█	█							
Perform the parameter test						█	█	█	█	█	█					
Perform the experiment analysis								█	█	█	█	█	█			
Complete of Test and Analysis											█	█	█			
Presentation Rehearsal													█			
SDP 11 Presentation														█		
Thesis Evaluation															█	█
Thesis Correction															█	█
Submission Final Proposal															█	█

Appendix D: GANTT CHART SDP 2

Appendix E

COST PROJECT

Item	Activity	Cost
Kitchen Blander (Pensonic,1.0L)	Grind food waste	RM 65.00
Food waste	Treatment process and lactic acid	RM 20.00
Lactic acid standard	Solution of lactic acid (500ml)	RM 80.00
HPLC	Observe lactic acid	RM 90.00/sample (x4)
Transportation	Buy the materials	RM 100.00
Total Costs		RM 625.00