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KINETIC ANALYSIS OF LACTIC ACID PRODUCTION FROM DIFFERENT FOOD WASTE SOURCES USING SOLID STATE FERMENTATION

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DECLARATION

I declare that this thesis entitled "(***Kinetic Analysis of Lactic Acid Production from different food waste sources using solid state fermentation,ssf)***" is the results of my own research except ascited in the references.

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Kinetic Analysis of Lactic Acid Production from different food waste sources using solid state fermentation (SSF).

ABSTRACT

This research project analyses the conversion of various food wastes, such as bread, vegetables, and dairy, into lactic acid by solid-state fermentation. It analyses lactic acid yields from various waste sources, investigates the kinetics of lactic acid production using the Monod equation, and examines the fermentation process in terms of waste type, substrate concentration, medium composition, and inoculum size. Results show that bread waste is the most efficient source of lactic acid production. The study investigates the relationship between lactic acid formation and process factors through kinetic analysis, with the aim of improving the fermentation process. Furthermore, it focuses on the need of using food waste for long-term lactic acid production, as well as the possibility for sugar reduction in this process. Overall, the study advances eco-friendly production methods and emphasizes the necessity of effective waste management in biotechnology.

Key word: substrate, lactic acid, solid state fermentation glucose reduction, food waste, lactobacillus

Analisis Kinetik Penghasilan Laktik Asid dari pelbagai sumber buangan makanan menggunakan fermentasi keadaan pepejal (SSF).

ABSTRAK

Kajian ini menganalisis pemprosesan pelbagai sisa makanan, seperti roti, sayur-sayuran, dan produk tenusu, menjadi asid laktik melalui fermentasi pepejal. Ia menganalisis hasil asid laktik daripada pelbagai sumber sisa, menyiasat kinetik penghasilan asid laktik menggunakan persamaan Monod, dan mengkaji proses fermentasi dari segi jenis sisa, kepekatan substrat, komposisi medium, dan saiz inokulum. Hasil kajian menunjukkan bahawa sisa roti merupakan sumber paling efisien dalam pengeluaran asid laktik. Kajian ini mengkaji hubungan antara pembentukan asid laktik dan faktor-faktor proses melalui analisis kinetik, dengan tujuan untuk meningkatkan proses fermentasi. Selain itu, kajian ini menumpukan kepada keperluan penggunaan sisa makanan untuk pengeluaran asid laktik jangka panjang, serta kemungkinan pengurangan gula dalam proses ini. Secara keseluruhan, kajian ini memajukan kaedah pengeluaran mesra alam dan menekankan keperluan pengurusan sisa yang berkesan dalam bidang bioteknologi.

Kata kunci: Senna alata, gelenggang, sebatian fenolik, pengekstrakan berbantukan gelombang mikro, komponen aktif

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LIST OF ABBREVIATIONS

LA	Lactic Acid
DW	Dairy waste
BW	Bread waste
VW	Vegetable waste
DNS	Dinitricsalli cylic acid
UV	Ultraviolet
UV-Vis	Ultraviolet-visible

LIST OF SYMBOLS

^{o}C	Celcius
g	gram
m	meters
mg	Milligram
ml	Mililiter
µg	microgram
µL	microliters
v/v	volume per volume

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Lactic acid (LA) is the common name for 2-hydroxypropanoic acid and is a highly versatile organic acid. It is used in the food, pharmaceutical, leather and textile industries and can also be used as a monomer for synthesis of polylactic acid. Food waste is a significant global challenge, with a stunning amount of food being wasted each year. This wastage not only poses environmental concerns but also results in the loss of valuable resources. At the same time, lactic acid is a crucial chemical compound with diverse industrial applications, including the production of biodegradable plastics, food additives, and pharmaceuticals. Utilizing food waste as a substrate for lactic acid production presents an attractive solution, as it addresses both waste management and the generation of a renewable resource. Solid-state fermentation (SSF) is a promising method for lactic acid production from food waste. SSF involves the cultivation of microorganisms on solid substrates, such as food waste, without the addition of excess water. This method offers several advantages, including low water usage, reduced energy requirements, and the potential to utilize a wide range of waste materials. To harness the full potential of SSF for lactic acid production, it is essential to understand the kinetics of the fermentation process. Kinetic analysis provides insights into the rate and efficiency of lactic acid production, allowing for the optimization of process parameters to maximize yield. By determining the kinetics, it becomes possible to identify the factors influencing lactic acid production and develop strategies to enhance the overall efficiency of the process.

Additionally, comparing the yield of lactic acid from different food waste sources is crucial for selecting the most suitable substrates. Different waste sources may vary in their composition, nutrient content, and availability, all of which can impact lactic acid production. By evaluating and comparing the yield from various food waste sources, it becomes possible to identify the most promising substrates for lactic acid production, thus facilitating efficient resource utilization and waste reduction.

Moreover, characterize the fermentation yield of lactic acid from different food waste sources is essential to understand the by-products generated during the process. By-products can affect lactic acid yield and quality, as well as the overall sustainability of the fermentation process.

Through a comprehensive analysis of the kinetics of lactic acid production, a comparison of yields from different food waste sources, and the characterize of fermentation yield, this research aims to contribute to the development of sustainable and efficient methods for lactic acid production from food waste sources. By addressing these objectives, the study will provide valuable insights for waste management practices, resource optimization, and the production of valuable chemicals from renewable sources.

1.2 Problem Statement

The problem statement for the research on kinetic modelling analysis of lactic acid production from different food waste sources using solid state fermentation could be as “Food waste is a significant global issue, contributing to environmental pollution and resource wastage. Despite the potential of food waste as a valuable resource for lactic acid production, there is a lack of comprehensive research on the kinetics, yield comparison, and fermentation yield characterize of lactic acid from different food waste sources using solid state fermentation. This knowledge gap delays the development of sustainable and cost-effective strategies for utilizing food waste to produce lactic acid. Therefore, there is a need to address this problem and conduct research to understand the kinetics of lactic acid production, compare the yield from different food waste sources, and characterize the fermentation yield. By doing so, we can solve the potential of food waste as a renewable resource and contribute to the development of environmentally friendly and economically viable lactic acid production processes.”

This problem statement highlights the key problem of underutilizing food waste as a valuable resource and the lack of research on lactic acid production from food waste using solid state fermentation. It emphasizes the need to bridge the knowledge gap and develop sustainable strategies for lactic acid production, aligning with the objectives of the research. By addressing this problem, the research can contribute to reducing food waste, minimizing environmental impact, and promoting the efficient use of resources in the production of lactic acid.

1.3 Research Objectives

The main aims for this study were to find out the lactic acid formation by reducing sugars. To further illustrate and guide the study, the following objectives was set:

i. To compare the yield of lactic acid from bread waste, vegetable waste and dairy waste using solid state fermentation.

ii. To determine the kinetics of lactic acid production from bread waste, vegetable waste and dairy waste using solid state fermentation.

iii. To determine the factors of fermentation yield of lactic acid from bread waste, vegetable waste and dairy waste.

1.4 Scope of Study

In the research of this study, the yield comparison of lactic acid from the three types of food waste was made is such a unique technique. This study will compare the yield of lactic acid obtained from bread waste, vegetable waste and dairy waste. This method involves fermenting each waste sources separately and quantifying the amount of lactic acid produced. For this, high-performance liquid chromatography and Ultraviolet-visible (UV-Vis) spectrophotometer was used to identify the peak area where it shows the highest amount of lactic acid production and the optical density to calculate the reducing sugar.

The second objective in this study is to determine the kinetic analysis of lactic acid production from bread waste, vegetable waste and dairy waste using solid state fermentation. This source has chosen based on the availability, composition, and potential for lactic acid production. In this, production of lactic acid by reducing sugar over time was monitored, analyses the growth of microorganisms, correlate the factors that affect the rate of lactic acid formation.

The final objective is to characterize the fermentation yield of lactic acid from different food waste sources. This involve investigating various parameters that affects the growth rate such as substrate concentration, and inoculum size that maximize the lactic acid yield and improve the efficiency of the process. Data of this research was compared with previous research. .

1.5 Significances of study

The study on comparing the yield of lactic acid from these three types of waste sources provides insights into economic viability, enabling the development of cost-effective production strategies. The kinetic analysis of lactic acid production using the solid-state fermentation method holds important implications. This addresses the global issue of food waste by utilizing as a renewable resource, contributing to waste reduction and resource optimization. Understanding the kinetics also facilitates process optimization, improving productivity and scalability. By doing the yield characterization it helps to assess the quality and suitability of lactic acid for various applications.

By gaining this knowledge, overall, this study promoted sustainable production methods by focusing on environmentally friendly method which is solid state fermentation. This contributes to waste reduction, sustainability, economic viability, application potential, and process optimization in lactic acid production.

CHAPTER 2

LITERATURE REVIEW

2.1 Food waste sources (Bread waste, Vegetable waste and Dairy waste)

Food waste is one of the largest municipal solid wastes, and most food waste ends up in landfills, causing significant economic losses and environmental concerns. In this study, we developed a fermentation process to convert food waste into bio renewable lactic acid and demonstrated that food waste is a superior feedstock for fermentation due to its embedded nutrients. Moreover, due to the embedded nutrients in food waste, the supplementation of yeast extract and peptone to fermentation can be reduced by over 50%, which can reduce the operating cost of lactic acid fermentation on an industrial scale. The choice of bread waste, vegetable waste, and dairy waste as the focus of this study is justified by their abundance, nutrient composition, and potential for lactic acid production through fermentation. In this experiment, bread waste is selected due to its prevalence in both domestic and commercial situations. It represents portion of food waste generated worldwide. From unsold loaves in bakeries to stale bread in households, bread waste offers a readily available and abundant source of carbohydrates. Carbohydrates serve as substrates for fermentation processes, making bread waste a desirable feedstock for lactic acid production. Vegetable waste is chosen because of its widespread occurrence throughout the food supply chain. Trimmed ends, peels, and spoiled vegetables constitute a considerable portion of food waste generated by households, supermarkets, and food processing facilities.

Vegetables are rich in sugars, starches, and other fermentable compounds, providing ample resources for microbial conversion into lactic acid. Additionally, the diverse nutrient profile of vegetable waste enhances the potential for lactic acid fermentation and promotes microbial growth and activity. Dairy waste is included due to the abundance of dairy products in the food industry and their propensity for spoilage. Expired milk, cheese trimmings, and whey by-products are common sources of dairy waste. These products contain lactose, a disaccharide sugar abundant in milk, which serves as a primary substrate for lactic acid bacteria during fermentation. Moreover, dairy waste offers a rich source of proteins, fats, and other nutrients that can support microbial growth and enhance lactic acid production. The selection of these three waste supports maximizing lactic acid production through fermentation. Each waste stream provides a diverse array of nutrients that can support microbial metabolism and facilitate the conversion of sugars into lactic acid. By utilizing these waste streams as feedstocks for fermentation, the study aims to demonstrate the feasibility of producing lactic acid from food waste while simultaneously addressing the pressing issue of food waste management.

2.2 Solid state fermentation

Solid-state fermentation (SSF) is a bioprocess that involves the cultivation of microorganisms on solid substrates, such as food waste, without the addition of excess water. This fermentation method offers several advantages over traditional submerged fermentation, including lower water requirements, reduced energy consumption, and the ability to utilize a wide range of solid waste materials as substrates. In the context of lactic acid production from different food waste sources, solid-state fermentation presents an attractive approach for sustainable and efficient utilization of these waste materials. Food waste, which accounts for a significant portion of the global waste stream, represents a valuable resource that can be harnessed through SSF to produce lactic acid, a versatile compound with various industrial applications. The kinetics of lactic acid production through SSF plays a crucial role in optimizing the fermentation process. Fermentation is a biochemical process of microorganism to produce different valuable products such as enzymes, hormones, biofuels etc. Fermentation process generally includes batch fermentation, fed batch fermentation and continuous fermentation. For enzyme production submerged and solid-state fermentation process is involved. Microorganisms utilize the nutrients present in the substrates for their growth and product synthesis. Change in chemical or physical environment highly effects the product formation and its quality and yield. These changes affect the growth and product synthesis kinetic leading to different quality yield of products. Thus, to ensure that the product formation is high quality and high yield, fermentation process must be monitored properly. Mathematical calculation and statistical analysis are needed to track the fermentation process and monitor the process for best results. This enhances the product quality as well as leads to high yield. By studying the kinetics, yield comparison, and fermentation yield characterization of lactic acid production through solid-state fermentation from different food waste sources, this research aims to contribute to the development of sustainable and efficient methods to produce lactic acid. Through the

optimization of fermentation parameters and a comprehensive understanding of the fermentation process, this study seeks to provide valuable insights for waste management practices, resource optimization, and the production of valuable chemicals from renewable sources.

2.3 Importance of lactic acid production

Lactic acid is a versatile compound that holds significant importance in various industries and applications. Its production from different food waste sources using solid state fermentation carries several advantages. Firstly, lactic acid has wide-ranging industrial applications. It is commonly used as a food additive, preservative, and flavouring agent. It serves as a precursor for the production of biodegradable plastics, which contribute to reducing environmental pollution caused by non-biodegradable materials. Lactic acid is also used in the pharmaceutical industry for the synthesis of drugs and as a component in cosmetic products. Therefore, studying the kinetics and yield of lactic acid production from food waste sources can provide insights into its potential applications and contribute to the development of sustainable and eco-friendly products.

Secondly, utilizing food waste as a substrate for lactic acid production promotes waste reduction and resource optimization. Food waste is a significant global issue, leading to environmental concerns and resource wastage. By converting food waste into lactic acid through solid state fermentation, the research contributes to waste management strategies and the efficient use of available resources. This approach not only reduces the volume of food waste destined for landfills but also harnesses its potential value as a renewable and cost- effective feedstock. Moreover, the comparison of lactic acid yields from different food waste sources provides valuable insights for industry and commercial applications. Identifying waste sources that yield higher amounts of lactic acid allows for the selection of more efficient and economically viable feedstocks. This knowledge can guide decision-making processes in industries involved in lactic acid production, promoting efficient resource allocation and cost-effective manufacturing practices.

In summary, the importance of lactic acid production from different food waste sources using solid state fermentation lies in its wide range of industrial applications, waste reduction and resource optimization, contribution to the circular economy, and potential for cost-effective manufacturing. By investigating the

kinetics, yield, and characterization of lactic acid production, the research enhances our understanding of this valuable compound and its sustainable production methods, thereby paving the way for innovative and environmentally friendly solutions in various industries.

2.4 Kinetic modelling analysis of lactic acid production based on monod equation

Kinetic approach was first approached by Luedeking (1958) in a homofermentative lactic acid production by Lactobacillus delbrueclcii. He found the formation of lactic acid depends both on growth and non-growth phase by a mathematical expression

$$dC/dtx\ dM/dt + y\ M$$

where C is product concentration, M is concentration of cell mass, t is time and x and y are parameters which are functions of pH in this system. Deindoerfer and Humphrey (1959) further modified the above reaction as:

$$x=0 \text{ (non-growth)}$$

$$dC/dt = y\ M\text{-}z\ dN/dt$$

$$y = 0 \text{ (growth)}$$

$$dC/dt\ x\ dM/dt=\text{-}2\ dN/dt$$

where N is the concentration of substrate or limiting nutrient concentration. Thus, lactic acid fermentation can be defined by two simple mathematical expressions (Deindoerfer, F.H).

Kinetic analysis of lactic acid production involves studying the rate of lactic acid formation and its relationship with various process parameters during fermentation. This analysis provides valuable insights into the dynamics of lactic acid production, allowing for process optimization and control. The kinetic analysis typically includes monitoring the concentration of lactic acid over time, using techniques such as high-performance liquid chromatography (HPLC) or enzymatic assays. By measuring the lactic acid concentration at different time points, the rate of lactic acid production can be determined.

The effects of key process variables on lactic acid production kinetics are also investigated. These variables may include temperature, pH, substrate concentration, inoculum size, and fermentation time. By systematically varying these parameters and analyzing their impact on lactic acid production, optimal conditions for maximum yield and productivity can be identified. Mathematical modelling and kinetic equations are often employed to describe the kinetics of lactic acid production. These models can help predict lactic acid production rates under different conditions and assist in process optimization and scale-up. It provides valuable information on the growth and metabolic activity of lactic acid bacteria, such as Lactobacillus species, and their ability to convert substrates into lactic acid. As in a cell's metabolism multiple biochemical reactions are occurring which includes enzymes and even production of these enzymes is also included in cell's metabolism. Thus, growth is calculated based on monod equation which is written as follows:

$$\mu = \frac{[s]}{\mu_{max}\, K_S + [S]}$$

μ= Specific growth rate of microorganism

$[s]$= Substrate concentration

μ_{max}= maximum specific growth rate

K_s= half saturation constant (substrate concentration)

2.5 Factors affecting kinetic parameters of microbial conversion of lactic acid

The kinetic parameters of microbial conversion of lactic acid can be influenced by various factors. Understanding these factors is crucial for optimizing the fermentation process and maximizing lactic acid production. Some key factors affecting the kinetic behaviors of microbial conversion of lactic acid are, Substrate Concentration. The concentration of the substrate, such as sugars or carbohydrates, affects the rate of lactic acid production. Higher substrate concentrations can lead to increased lactic acid production initially, but excessive substrate levels may inhibit microbial growth and fermentation. Next, ph. Of fermentation medium plays a significant role in microbial activity and lactic acid production. Different microorganisms have varying pH optima for lactic acid production. It is essential to maintain the pH within the suitable range to support microbial growth and optimize lactic acid production. Third is temperature where it directly influences the growth rate and metabolic activity of microorganisms. Different microorganisms have different temperature optima for lactic acid production. Controlling and maintaining the appropriate temperature is vital to ensure optimal microbial growth and lactic acid yield. Then, microbial strain used in the fermentation process can greatly impact lactic acid production. Different strains of lactic acid bacteria exhibit variations in their metabolic capabilities and growth characteristics, leading to differences in the rate and yield of lactic acid production.

2.6 Lactic acid recovery from industrial waste

Lactic acid recovery from industrial waste is a process aimed at extracting and purifying lactic acid from waste streams generated by industrial operations. Industrial waste sources, including by-products from food processing, agricultural waste, and fermentation residues, contain lactic acid that can be recovered and utilized. The recovery process involves several steps, starting with the collection of waste streams containing lactic acid. Pre-treatment steps may be necessary to remove impurities and contaminants from the waste stream.

Acidification is performed to lower the pH of the waste stream and convert lactic acid to its acidic form, facilitating its separation. Various separation techniques such as solvent extraction, ion exchange, membrane separation, or precipitation methods are employed to isolate lactic acid from the waste stream. Purification steps follow, eliminating remaining impurities and by-products.

Concentration of the purified lactic acid can be achieved through processes like evaporation or reverse osmosis. The recovered lactic acid can then undergo further processing and formulation to meet specific market requirements, such as transforming it into liquid solutions, salts, or esters.

Lactic acid recovery from industrial waste offers benefits such as waste reduction, resource optimization, and the development of a circular economy. It provides a sustainable and cost-effective source of lactic acid, which finds applications in various industries including food and beverages, pharmaceuticals, cosmetics, and bioplastics

Overall, lactic acid recovery from industrial waste contributes to waste reduction, resource optimization, and the development of a sustainable and circular economy. By transforming waste into valuable products, it promotes environmental sustainability and economic viability.

2.7 Composition of food waste

The composition of various food waste sources plays a vital role in the kinetic analysis of lactic acid production using solid state fermentation. The composition of food waste determines the availability of nutrients and substrates for microbial growth and lactic acid production. Here is an explanation of the composition of various food waste sources and its relevance to the kinetic analysis. Protein content in food waste sources contributes to the availability of amino acids for microbial metabolism. Microorganisms utilize proteins as a source of nitrogen for growth and enzyme production. Higher protein content can potentially provide more nitrogenous compounds, supporting microbial activity and lactic acid production. Carbohydrates, such as sugars and starches, serve as the primary energy source for microbial fermentation.

They are broken down by enzymes produced by microorganisms into simpler sugars, which are further metabolized to produce lactic acid. Higher carbohydrate content in food waste sources can provide more fermentable substrates, promoting lactic acid production. Vitamins act as essential cofactors for various enzymatic reactions in microbial metabolism. They are required for the growth and metabolic activities of microorganisms. Food waste sources with a higher vitamin content can support optimal microbial growth and enhance lactic acid production. Fats present in food waste sources can serve as a source of energy for microbial growth and metabolism. Some microorganisms have the ability to metabolize fats and convert them into lactic acid. However, excessive fat content may negatively affect microbial growth and fermentation efficiency. Starch, a complex carbohydrate, needs to be hydrolysed into simple sugars before microbial fermentation. Microorganisms produce amylase enzymes to break down starch into fermentable sugars like glucose. Food waste sources rich in starch can provide a substantial substrate for microbial conversion to lactic acid.

Food source	Protein	Carbohydrates	Vitamins	Fats	Starch
Vegetable waste	0.5 % - 2%	5 % - 15 %	0.1 % to 2 %	< 0.5 %	3% - 20 %
Dairy waste (milk)	10 % - 25 %	1 % - 10 %	0.1 % to 5 %	60 % – 90 %	0.5 – 10 %
Cereal waste (Bread)	5 % - 15 %	60 % - 80 %	0.1 % to 2 %	1 % to 5 %	30 % to 60 %

Table 1 shows Composition of food waste sources

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

The materials used in this study are bread waste, vegetable waste and dairy waste. The sample were collected from cafe and restaurants at Jeli, Kelantan. Approximately 500 gram of waste sample was collected for this study.

Figure 1: Bread waste sample

Figure 2: Vegetable waste sample

Figure 3: Dairy waste sample

3.2 Chemicals

The chemicals and reagent needed for lactic acid production and reducing sugar are outlined in Table 2.

Table 2: The list of chemicals used in this research.

Chemicals
Dinitrosalicylic acid
Distilled water
Sodium hydroxide
Phosporic acid
Sodium potassium tartarate
Acetonitrile HPLC Grade
Amylase enzyme
Deionized water
Glucose
LAB Culture
Nutrient agar
MRS Broth

3.3 Apparatus and Equipment

The apparatus and equipment used in this experiment are summarized Table 2.1 and Table 2.2

Table 2.1: List of apparatus used in the research.

Apparatus and Equipment
Beaker (50mL,250mL & 500mL)
Measuring cylinder (10mL & 100mL)
Test tube
Test tube rack
Zip lock plastic bag
Aluminium foil

Disposable dropper
Clear reagent bottle
Micropipette
Centrifuge tube
Centrifuge Tube rack
Spectrophotometer cuvettes
Pipette tips (1000μL)
Digital thermometer
Electronic balance
Centrifuge microtube
Gloves
HPLC vial
Parafilm
Funnel
Filter paper
Disposable Syringe
Syringe Filter
Mask
Reagent bottle (250mL)
Sieve net (500 μm)

Table 2.2: List of equipment used in the research

No	Equipment	Purpose
1	Blender machine	Particle size determination of samples
2	Centrifuge	Separation of sample from precipitate
3	Ultraviolet-visible (UV-Vis) spectrophotometers	Measuring light absorbance across the ultraviolet and visible ranges of the electromagnetic spectrum
4	High-Performance Liquid Chromatography (HPLC)	to separate, identify, and quantify the components of a mixture.
5	Incubator shaker	For continuous stirring of substrates
6	Water bath	To heat up the DNS Reagent
7	Oven	To dry the samples

3.4 Method

3.4.1 Preparation of food waste sample

Around 500g of each bread, vegetable and dairy food waste were collected from restaurants around, Jeli Kelantan. After that, the sample was rinsed a little using tap water to remove non- biodegradable materials and dried the sample for 3 hours in the oven. The sample drying process was performed to remove the water content of the sample to obtain the exact amount of sample before the next process.

After that, the dried sample were taken to the laboratory to be ground into powder form. The grinding process is done using a blender machine. Next, the powder sample was weighed, andstored in zip-lock bags before further use.

3.4.2 Agar and broth preparation

Dissolved 28 grams of nutrient agar powder in 1 liter of distilled water. Stirred well to ensure complete dissolution. Then, transferred the nutrient agar solution to a sterilization stock bottle and autoclave at 121℃ for15 minutes to sterilize the medium. Once done with autoclave, allowed the agar to cool to approximately 45-50 ℃, by ensuring it is still in a liquid state but not too hot to handle. Once cooled, nutrient a solution was poured into the prepared and sterilized petri dish and kept drying till become solidify. For the broth, MRS Broth was used where 52 grams of MRS Broth was suspended in 1 liter of distilled water. Stirred well to ensure complete dissolution. Then, the broth was transferred to a sterilized stock bottle and autoclaved at 121 ℃ for 15minutes. This is to ensure the medium is sterilized. Once done with autoclave, after cooling 50 ml of broth was transferred to test tubes.

3.4.3 Colony isolation and observation

This is where the Lactobacillus strain were used. Used a sterile inoculating loop, dipped the loop in the strain then proceeded to streak it onto a fresh agar plate to obtain the pure culture. This is known as streak plate technique. Then, after 24- 48 hours of incubation the pure culture was obtained. After incubation, observed the agar plate for the presence of colonies. Lactobacillus colonies appeared as small, round, and usually creamy-white or off-white in colour.

3.4.4 Inoculum and enzyme preparation

The selected microbial strain, Lactobacillus spp. has been cultivated in MRS Broth where single colony was picked around 5 to 10 % colony. The cultures have been incubated for an appropriate duration, 24 to 48 hours, at a temperature of 37°C. This to ensure the growth and viability of the microbial strains, generating healthy and active inoculum for the subsequent fermentation process. For the enzyme, amylase enzyme was used. Each 50 ml, 1g of enzyme were used.

3.4.5 Preparation of standard stock solution for glucose

For the glucose standard curve, in this experiment DNS assay method was used. To prepare the 200 ml of DNS reagent, 1 g of DNS was added and mix up with 30 ml of water. 30 g Sodium potassium titrate, 20 g sodium hydroxide was mixed along with 170 ml of distilled water. Then, reagent was water bath at 95 ℃. Next, for the glucose stock solution, 1 g of glucose was added to 100 ml of distilled water. Pipette out 0.2,0.4,0.6,0.8 and 1ml of standard glucose solution and transferred into test tubes and make it up to 2ml with distilled water. Then, 2ml prepared DNS reagent was added to the test tube. Prepared blank solution where 2ml of distilled water with 2 ml of DNS reagent.

3.4.6 Solid state fermentation set up and sample collection

For the solid-state fermentation set up, 250 ml conical flasks were wrapped with cotton wool and then capped with aluminum foil. Proceeded with autoclave to ensure it is sterilized. Then, substrates, enzyme and Lactobacillus culture were added into it, all for 200 ml. Samples were in incubator shaker at 37℃ 150 rpm. Then, samples were collected at 8.30 am and 4.30 pm for 5 days continuously.Same process repeated for only dairy waste with varying substrate concentration which is 30 ml, 60 ml, and 90 ml.

3.4.7 Glucose analysis using UV-VIS spectrophotometer

To the glucose UV-Vis spectrophotometer was used. This is where, collected sample was centrifuged and 1ml of sample was added to a test tube along with 1ml of DNS reagent in each sample. Then the sample was heated up at 70℃ for 5 minutes, color changes will occur, cool down and check absorbance and optical density value at 540nm by using UV-Vis Spectrophotometer.

3.4.8 Determine the fermentation yield

The lactic acid produced during fermentation has been determine in terms of its media composition,inoculum size and type and substrate concentration. This was done by comparing other research papers and data from high performance liquid chromatography.

3.4.9 HPLC analysis for lactic acid formation

The standard solution of lactic acid was used in this study. The lactic acid was the standard solution for the estimation of the lactic acid formation. Commercial lactic acid (85 %) was purchase from Sigma Aldrich. 1000 mg/mL was prepared from the commercial lactic acid with acetonitrile HPLC grade as a solvent. Then, different concentrations of 100, 200, 400 and 600 (mg/mL) were

prepared for a standard curve of lactic acid to be used for HPLC Analysis. The calibration curve was plotted, and the lactic acid formation was calculated. The lactic acid formation in the substrate was determined using the HPLC method. For sample preparation firstly the sample was centrifuged at 10000 rpm, then the sample was pipette out and diluted with acetonitrile hplc grade. According to the manufacturer's instructions filter the sample using a 0.45µm syringe filter any suitable filtration method to remove any insoluble particles or impurities that mayinterfere with the HPLC analysis before injection into the HPLC system then setted up and prepared the HPLC instrument.

CHAPTER 4

RESULTS AND DISCUSSION

This chapter discuss the results of the, reducing sugar using UV-Vis spectroscopy analysis and HPLC analysis for the lactic acid formation, kinetic modelling by using Monod equation and also the yield comparison.

4.1 Standard lactic acid using High Performance Liquid Chromatography (HPLC)

Table 3 shows the standard reading of lactic acid with concentrations at 100, 200, 400 and 600 mg/mL and measured using high performance liquid chromatography (HPLC). The calibration curve of the lactic acid standard is plotted as shown in Figure 5. The R^2 of the calibration curve is 0.983, meaning that the calibration curve for the lactic acid standard is significant. The equivalent equation obtained from the graph is y = 105106x + 2E+07 and it is used to calculate the unknown concentration of lactic acid from bread waste, vegetable waste and dairy waste.

Table 3: HPLC analysis of standard Lactic Acid

Peak	Ret. Time	Area	Height	Area %	Height %
1	2.443	61856070	2684161	97..217	94.242
2	3.426	137577	28926	0.216	1.016
3	3.692	57916	8831	0.091	0.310
4	3.843	46106	4188	0.072	0.147
5	4.147	55600	4266	0.087	0.150
6	4.461	109722	7676	0.172	0.270
7	4.789	141014	100260	0.222	0.346
8	5.044	1222523	2848164	1.921	3.520

Table 3.1: Reading of lactic acid standard.

Concentration (mg/mL)	Peak Area/AU
100	24574379
200	40593355
400	61856070
600	78063235

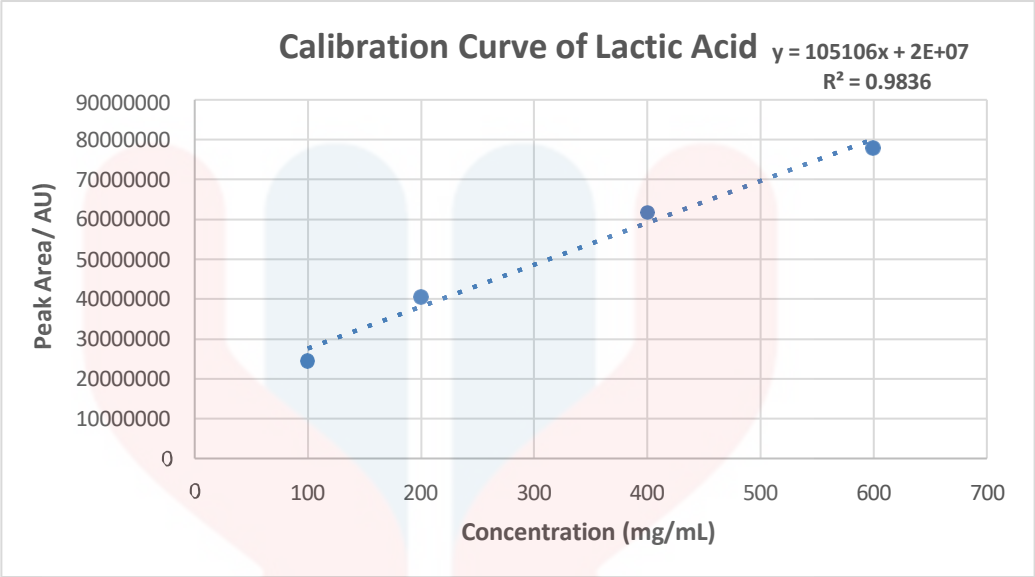


Figure 4: Calibration curve of Lactic Acid Concentrations used 100,200,400,600 mg/mL and the absorbance measured at 200 nm.

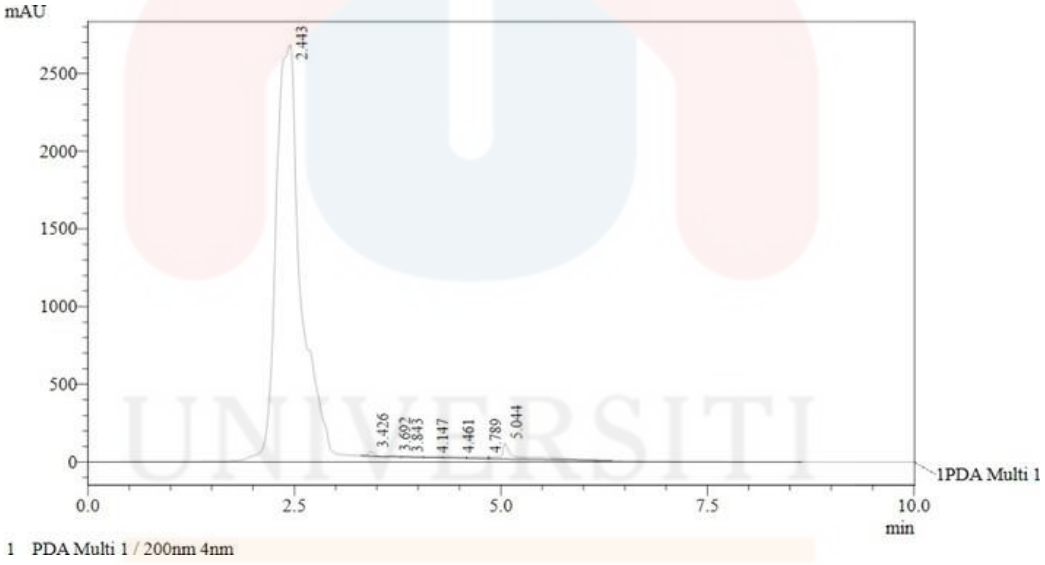


Figure 4.1: HPLC chromatograms of standard lactic acid

A calibration curve is a graphical representation of the relationship between the concentration of an analyte and the response of the analytical instrument. In the case of HPLC, the concentration of lactic acid is plotted against the corresponding peak area obtained from the chromatogram. The curve serves as a tool for quantification by allowing the conversion of instrument response to concentration.

To construct the calibration curve for lactic acid, a series of standard solutions with 100,200, 400, 600 mg/mL concentrations are prepared. These solutions are then injected into the HPLC system, and the resulting chromatograms are analyzed. The peak areas corresponding to each concentration are recorded, and a linear regression analysis is performed to generate the calibration curve. Several factors can influence the construction and accuracy of the calibration curve. These include the choice of mobile phase, column type, flow rate, and detector wavelength (Hefnawy et al., 2006). Optimization of these parameters is crucial to achieving a stable and reproducible chromatographic separation.

The calibration method for lactic acid can be applied to analyze lactic acid production. The calibration curve of lactic acid using HPLC is a vital tool in analytical chemistry, providing a quantitative method for the accurate determination of this lactic acid. The optimization of HPLC parameters, validation of the method, and careful attention to accuracy and precision contribute to the reliability of the calibration curve, making it a valuable asset in both research and industrial settings.

4.1.1 Lactic acid formation of food waste sources by HPLC analysis

HPLC analysis results were obtained for lactic acid calibration curve and identification of lactic acid from food waste sources. HPLC is known for its rapid analysis, providing fast results without compromising data quality. This efficiency is invaluable in high-capacity laboratories, where the analysis of many samples is a routine part of the workflow.

The food waste source sample were analyzed using HPLC with the method described previously. A 5 µL sample was injected into the HPLC system, and the PDA was set at 200 nm. The lactic acid in food waste samples were detected by comparing the peak retention time and peak area.

The retention time for lactic acid in HPLC chromatograms is a critical parameter for identifying and quantifying compounds. This technique offers a higher level of

resolution. HPLC generally offers higher sensitivity and lower detection limits. This is crucial, especially when dealing with samples with lower analyte concentrations. HPLC provides a more detailed and accurate analysis, especially in complex samples. The separation capability of HPLC reduces interferences and enhances precision.

Table 4 : Result of HPLC analysis on lactic acid formation based on dairy waste

Sample	Time	Average Ret. Time	Average Peak Area	Average Height	Average Area %
Day 1	8.30 am	2.3805	11102203	211433	30.07
	4.30 pm	2.4985	9556330	236,396.5	24.233
Day 2	8.30 am	2.258	17504684.5	276,891	39.1145
	4.30 pm	2.292	11954603	226,714	28.062
Day 3	8.30 am	2.303	13435417.5	252,748.5	32.3
	4.30 pm	2.302	19961835	381,863	41.1795
Day 4	8.30 am	2.32	20437197.5	329,662.5	41.0615
	4.30 pm	2.325	11290,366.5	277,063.5	26.802
Day 5	8.30 am	2.4155	23064221.5	465,087	49.1875
	4.30 pm	2.338	5423,930.5	257,429	13.1845

Based on the table 3.2, provided for the HPLC analysis on lactic acid formation based on dairy waste, several trends emerge. The retention time for lactic acid in the dairy waste samples ranges from approximately 2.258 to 2.4155 minutes across the different days and times of analysis. Both the average peak area and height exhibit variations across the different samples. The highest peak value in a lactic acid HPLC chromatogram indicate the highest concentration of lactic acid present in the sample at that retention time. It represents the maximum amount of lactic acid detected by the chromatographic analysis at the given conditions. The highest peak value likely corresponds to the sample with the highest concentration of lactic acid for dairy waste is on day 5 8.30 am, with the peak area 23,064,221.5. This shows that at 8.30 am on day 5, the sample contained the highest concentration of lactic acid among all the samples analyzed, compared to other days and times. Fluctuations in the area percentage, representing the proportion of lactic acid in the overall chromatogram, are also notable, with the highest percentage observed on Day 5 at 8.30 am. Across the five days of analysis, there appears to be some variability in the peak values. However, Day 5 consistently shows higher values compared to other days, suggesting a potential accumulation or increase in lactic acid formation in the dairy waste over time. Additionally, there is a noticeable difference in peak values between morning (8.30 am) and afternoon (4.30 pm) analyses, indicating a possible influence of the time of analysis on lactic acid formation.

Table 4.1: Result of HPLC analysis on lactic acid formation based on vegetable waste.

Sample	Time	Average Ret. Time	Average Peak Area	Average Height	Average Area %
Day 1	8.30 am	2.2485	15542722	432959.5	28.1415
	4.30 pm	2.3165	25030504.5	632,285	38.8925
Day 2	8.30 am	2.2575	21318925.5	484,240	39.4995
	4.30 pm	2.28	30946531	651,474.5	49.653
Day 3	8.30 am	2.2045	20750370	344603.5	47.0875
	4.30 pm	2.237	27314862.5	460,465	50.472
Day 4	8.30 am	2.948	10268392.5	284,860.5	17.7
	4.30 pm	2.3005	18227199.5	388,556	39.6535
Day 5	8.30 am	2.881	6253947	232,900	12.4345
	4.30 pm	2.282	9200622.5	299,254	23.501

Based on the table 3.3, provided for the HPLC analysis on lactic acid formation based on vegetable waste, several trends can be observed. The retention time for lactic acid in the vegetable waste samples ranges from approximately 2.2045 to 2.948 minutes across the different days and times of analysis. Similarly, to the dairy waste analysis, both the average peak area and height exhibit variations across the different samples. Higher value observed on Day 2 at 4.30 pm the highest peak value likely corresponds to the sample with the highest concentration of lactic acid, which was obtained on day 2 itself, at 4.30 pm with the peak area 30,946,531. This shows that at 4.30 pm on day 2, the sample contained the highest concentration of lactic acid among all the samples analyzed compared to other days and times. Fluctuations in the area percentage, representing the proportion of lactic acid in the overall chromatogram, are also notable, with the highest percentage observed on Day 4 at 4.30 pm. Across the five days of analysis, variability in the peak values is clear. Day 2 at 4.30 pm consistently shows higher values compared to other days, indicating a potential accumulation or increase in lactic acid formation in the vegetable waste over time. Additionally, there is a visible

difference in peak values between morning (8.30 am) and afternoon (4.30 pm) analyses, suggesting a possible influence of the time of analysis on lactic acid formation.

Table 4.5: Result of HPLC analysis on lactic acid formation based on bread waste.

Sample	Time	Average Ret. Time	Average Peak Area	Average Height	Average Area %
Day 1	8.30 am	2.323	6030,888	188,1455.5	16.4715
	4.30 pm	2.373	11410,068.5	204,928	29.4755
Day 2	8.30 am	2.281	5187,290.5	309,619.5	10.9855
	4.30 pm	2.3125	20261955	603,426.5	36.2225
Day 3	8.30 am	2.335	15412,837.5	249,147.5	36.5915
	4.30 pm	2.2185	14577865	220,932.5	37.5115
Day 4	8.30 am	2.3605	11445,391	206,001.5	28.6735
	4.30 pm	2.286	13419838	299,736	28.2255
Day 5	8.30 am	2.3275	9207511.5	193,912.5	24.8795
	4.30 pm	2.3035	11804019.5	280,748.5	26.7805

Based on the table 3.4, provided for the HPLC analysis on lactic acid formation based on bread waste, several trends can be observed. The retention time for lactic acid in the bread waste samples ranges from approximately 2.2185 to 2.373 minutes across the different days and times of analysis. Similarly, to the previous analyses, both the average peak area and height exhibit variations across the different samples. The highest peak value likely corresponds to the sample with the highest concentration of lactic acid, which was obtained on Day 2 at 4.30 pm, with the peak area of 20,261,955. This indicates that at 4.30 pm on Day 2, the sample contained the highest concentration of lactic acid among all the samples analyzed compared to other days and times. Fluctuations in the area percentage, representing the proportion of lactic acid in the overall chromatogram, are also notable, with the highest percentage observed on Day 3

at 8.30 am. Across the five days of analysis, variability in the peak values is evident. Day 2 at 4.30 pm consistently shows higher values compared to other days, indicating a potential accumulation or increase in lactic acid formation in the bread waste over time. Additionally, there is a noticeable difference in peak values between morning (8.30 am) and afternoon (4.30 pm) analyses, suggesting a possible influence of the time of analysis on lactic acid formation.

4.2 Screening of reducing sugar of standard glucose using UV-Vis spectroscopy analysis

A glucose standard curve is an important tool in analytical chemistry, providing a reliable method for quantifying the concentration of glucose in various samples. To construct the curve, a series of standard solutions with known concentrations of glucose are prepared and analyzed using a spectrophotometer to measure their absorbance values at a specific wavelength, around 540 nm. These absorbance values are plotted against the corresponding concentrations of glucose, forming a linear relationship. This curve serves multiple purposes, including quantitative analysis of unknown samples by comparing their absorbance values to the standard curve, ensuring the accuracy and precision of spectrophotometric measurements, calibrating instruments for accurate readings, and validating analytical methods for glucose quantification. Overall, the glucose standard curve is a for ensuring the reliability and validity of glucose concentrations.

Table 5 : Reading of glucose standard.

Tube	Concentration	Absorbance
1	0	0
2	0.2	0.839
3	0.4	1.268
4	0.6	1.733
5	0.8	2.032
6	1	2.539

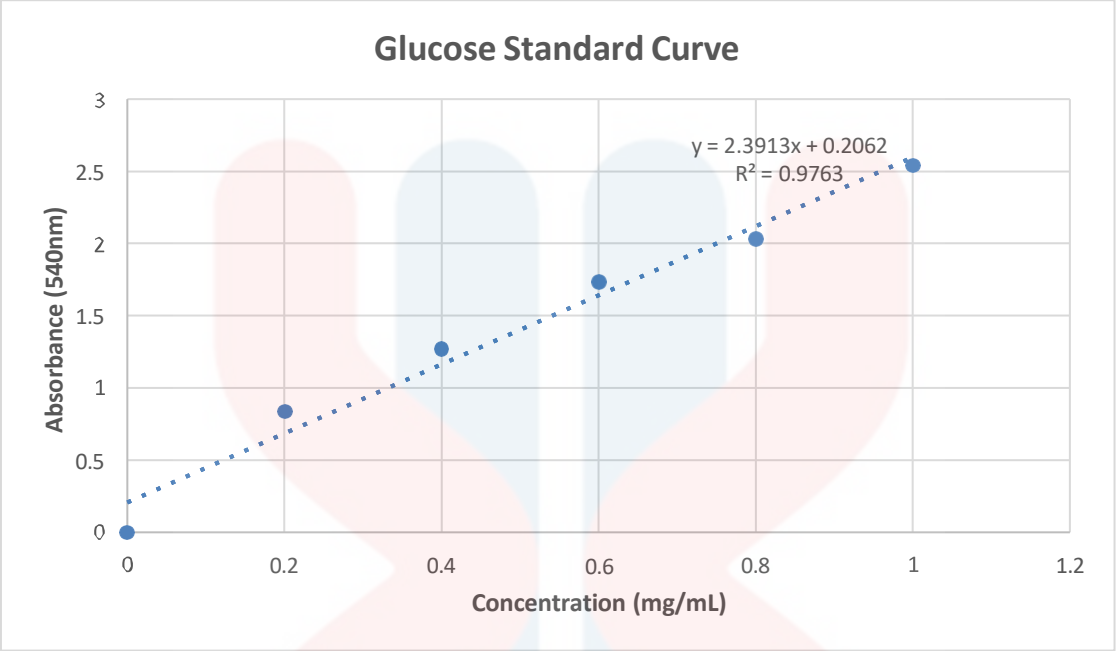


Figure 5 : Standard calibration curve of glucose.

Table 4 shows the readings of glucose standard with the concentration of 0, 0.2, 0.4, 0.6, 0.8, 1 mg/mL and measured by using UV-Vis spectrophotometer. The calibration curve of glucose standard is plotted as shown in Figure 7 R^2 of the calibration curve is 0.9763, means the calibration curve for the glucose standard was significant. The equivalent equation obtained from the graph is $y = 2.3913x + 0.2062$ and it is used to calculate the concentration reducing sugar.

4.2.1 Screening of reducing sugar of bread waste, vegetable waste and dairy waste using Uv-Vis analysis

Table 5.1 : Result of reducing sugar for bread, vegetable and dairy waste.

Days	Time	Bread waste	Vegetable waste	Dairy waste
Day 1	8.30 am	1.01601	1.127	1.674
	4.30 pm	0.960	0.664	1.259
Day 2	8.30 am	0.894	0.373	1.100
	4.30 pm	0.865	0.322	1.050
Day 3	8.30 am	0.714	0.306	1.025
	4.30 pm	0.675	0.237	1.010
Day 4	8.30 am	0.370	0.217	0.991
	4.30 pm	0.308	0.195	0.973
Day 5	8.30 am	0.273	0.137	0.918
	4.30 pm	0.231	0.130	0.886

Figure 5.2 shows reducing sugar for dairy waste

Fermentation time	Concentration (g/ml)
0	0.849412453
8	0.786266884
16	0.612721114
24	0.546230084
40	0.525320955
48	0.514866391
64	0.508593652
72	0.500648183
88	0.493120897
94	0.470120855
110	0.456739012

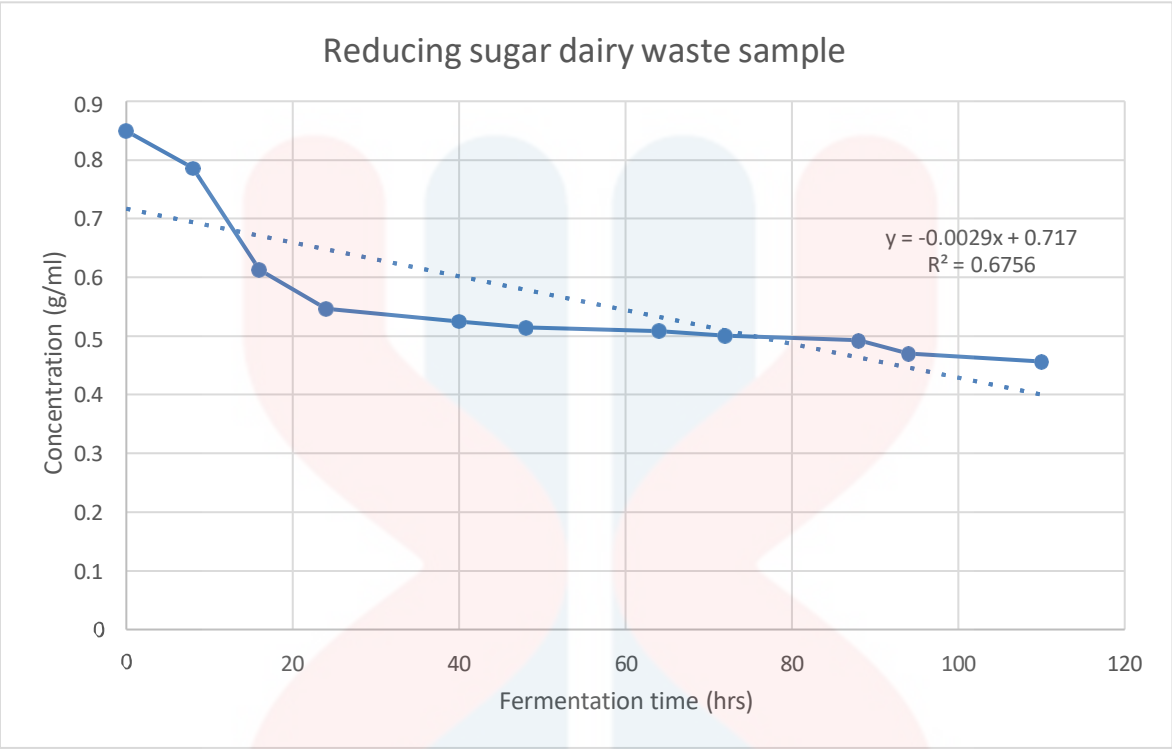


Figure 5.1 shows reducing sugar for bread waste

Table 5.3 shows fermentation time with concentration

Fermentation time	Concentration (g/ml)
0	0.660812111
8	0.511102747
16	0.487684523
24	0.460084473
40	0.447957178
48	0.3848116
64	0.368502488
72	0.240956802
88	0.215029482
94	0.200393092
110	0.182829423

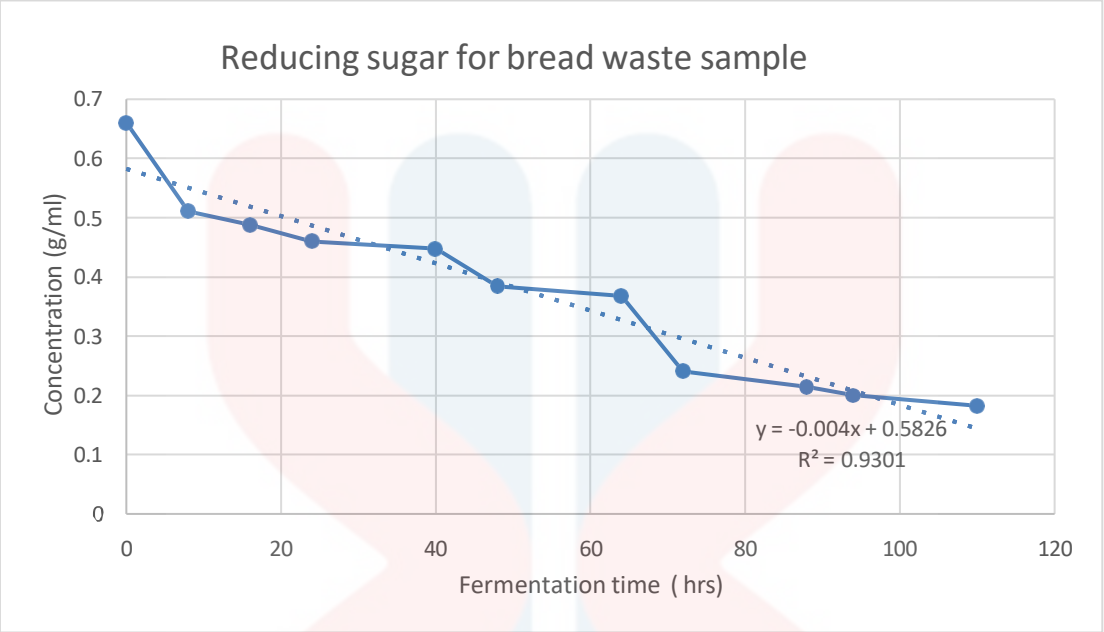


Figure 5.2 shows reducing sugar for vegetable waste

Table 5.4 shows fermentation time with concentration

Fermentation time	Concentration (g/ml)
0	0.639484799
8	0.557521014
16	0.36390248
24	0.242211349
40	0.220884038
48	0.214193117
64	0.185338519
72	0.176974867
88	0.16777485
94	0.143520261
110	0.140592983

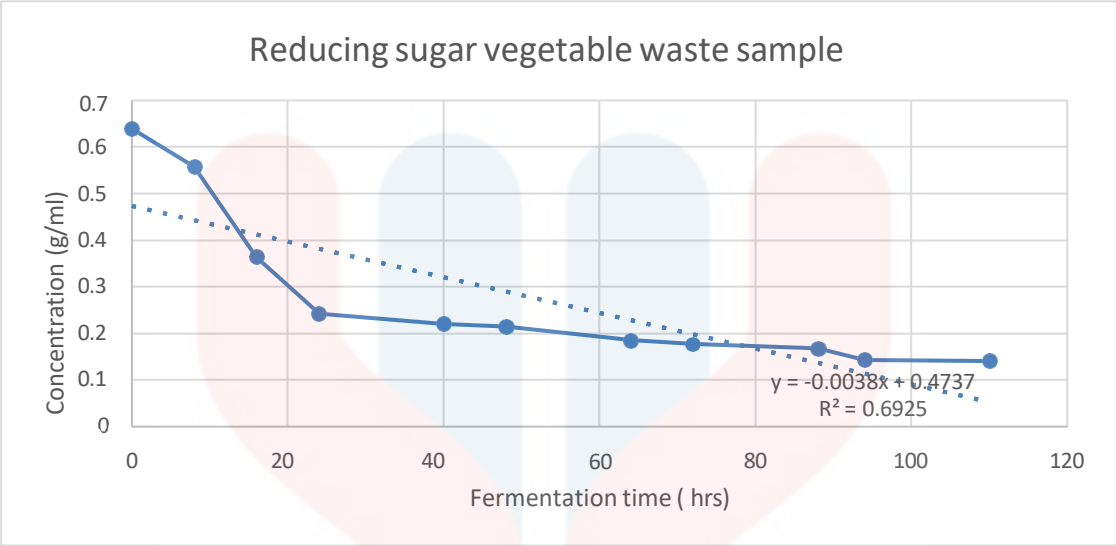


Figure 5.3 shows reducing sugar for vegetable waste

Based on the table 4.1, the optical density (OD) values provided in the table represent the concentration ofreducing sugars present in the samples of bread waste, vegetable waste, and dairy waste. Reducing sugars arecarbohydrates that can reduce chemicals measured using method, which is DNS (3,5-dinitrosalicylic acid) assay, which relies on the reduction of DNS by reducing sugars to form a coloredproduct that can be quantified spectrophotometrically. Across all waste types, there is a trend of decreasing OD values over the course of the five days of analysis. For bread waste, the OD values range from 1.016 onDay 1 at 8.30 am to 0.231 on Day 5 at 4.30 pm. Similarly, vegetable waste exhibits a decrease in OD valuesfrom 1.127 on Day 1 at 8.30 am to 0.137 on Day 5 at 8.30 am. In contrast, dairy waste consistently showshigher OD values compared to the other waste types, with values ranging from 1.674 on Day 1 at 8.30 amto 0.886 on Day 5 at 4.30 pm. While all waste samples successfully reduce the sugar, based on theprovided data, dairy waste demonstrates the highest concentration of reducing sugars. The highest OD valueobserved in the data is 1.674, which corresponds to dairy waste on Day 1 at 8.30 am. Conversely, the lowestOD value is 0.231, observed in bread waste on Day 5 at 4.30 pm. Therefore, based on this data, dairy wasteappears to have the highest concentration of reducing sugars among the three waste types analyzed, whilebread waste exhibits the lowest concentration.

4.3 Yield comparison of lactic acid formation by reducing sugar based on different food waste sources

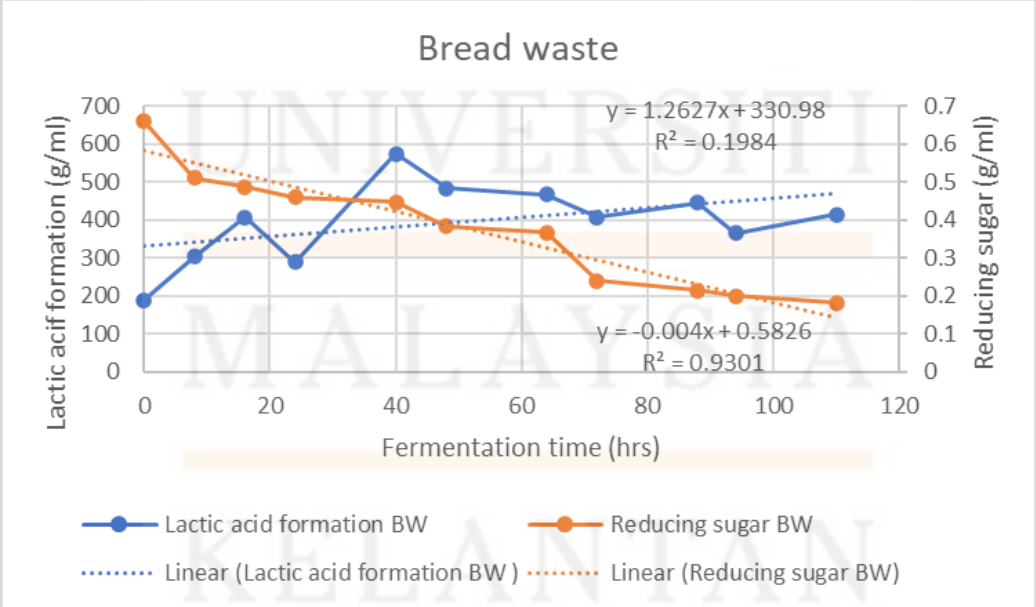
The yield coefficient, obtain as a percentage, is a parameter used to assess the efficiency of a fermentation process in converting a substrate reducing sugars into a desired product which is lactic acid. Lactic acid formation from reducing sugars derived from different food waste sources, the yield coefficient shows how effective each food waste source contributes to lactic acid production. A higher yield percentage indicates a more efficient conversion of reducing sugars into lactic acid, while a lower yield percentage suggests less efficient conversion. By comparing the yield coefficients obtained from different food waste sources, can evaluate which waste source yields the highest percentage of lactic acid relative to the amount of reducing sugar consumed. This comparison is to identify the most suitable food waste source for lactic acid production, optimizing fermentation conditions, and potentially improving process efficiency. The yield coefficient provides a quantitative measure of the efficiency of lactic acid formation from reducing sugars derived from various food waste sources.

4.3.1 Yield for bread waste

Table 6 shows fermentation time for lactic acid formation by reducing sugar for bread waste

Fermentation time	Lactic acid formation BW	Reducing sugar BW
0	190.2840942	0.660812111
8	305.0423002	0.511102747
16	407.399549	0.487684523
24	288.9899815	0.460084473
40	575.8368694	0.447957178
48	483.5658764	0.3848116
64	467.6776778	0.368502488
72	408.07168	0.240956802
88	445.6422659	0.215029482
94	365.4883927	0.200393092
110	414.8958099	0.182829423

Figure 6 shows lactic acid formation by reducing sugar of BW

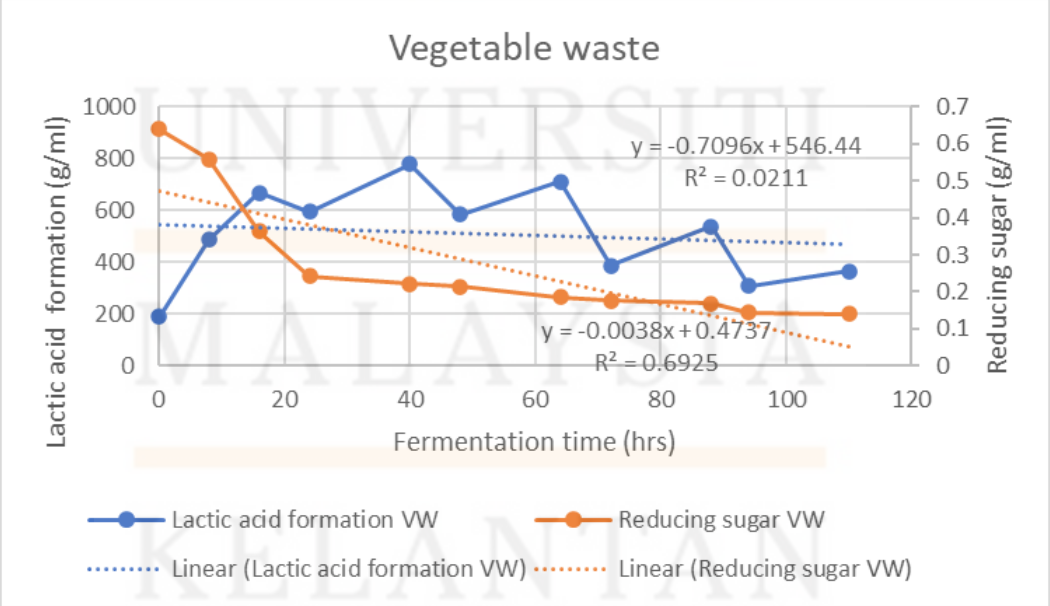


4.3.2 Yield for vegetable waste

Table 6.1 shows fermentation time for lactic acid formation by reducing sugarfor vegetable waste

Fermentation time	Lactic acid formation VW	Reducing sugar VW
0	190.2840942	0.639484799
8	486.0373718	0.557521014
16	666.5747816	0.36390248
24	595.9493369	0.242211349
40	779.147356	0.220884038
48	585.13063	0.214193117
64	710.0424809	0.185338519
72	385.6752707	0.176974867
88	537.1187087	0.16777485
94	309.2867581	0.143520261
110	365.357306	0.140592983

Figure 6.1 shows lactic acid formation by reducing sugar of VW

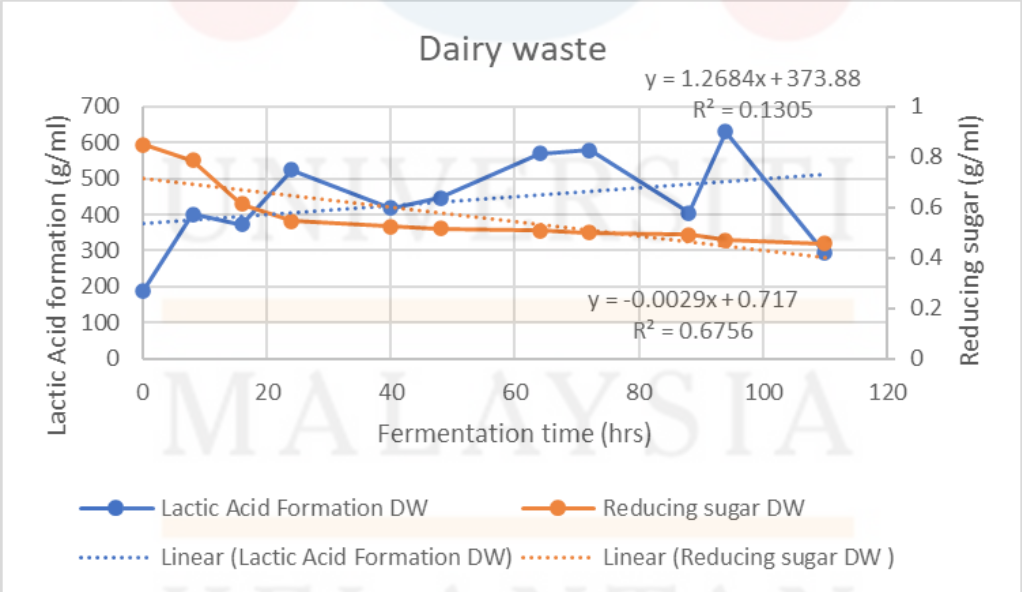


4.3.3 Yield for dairy waste

Table 6.2 shows fermentation time for lactic acid formation by reducing sugarfor dairy waste

Fermentation Time	Lactic Acid Formation DW	Reducing sugar DW
0	190.2840942	0.849412453
8	401.5413582	0.786266884
16	372.1258539	0.612721114
24	523.3703404	0.546230084
40	417.7611744	0.525320955
48	445.938719	0.514866391
64	570.1260632	0.508593652
72	579.1714555	0.500648183
88	405.1218104	0.493120897
94	629.1595437	0.470120855
110	293.4928643	0.456739012

Figure 6.2 shows lactic acid formation by reducing sugar of DW



Different types of food waste sources were used in this experiment. Dairy wastes were analyzed for their potential to release reducing sugar. The maximum reducing sugars of 0.457 g were released from dairy waste

at 110 hours of fermentation time after simultaneous saccharification which is on the 5th day. Bread wastes were analyzed for their potential to release reducing sugar. The maximum reducing sugars of 0.141 g were released from vegetable waste at 110 hours of fermentation time after simultaneous. saccharification which is on the 5th day.

Bread wastes were analyzed for their potential to release reducing sugar. The maximum reducing sugars of 0.183 g were released from dairy at 110 hours of fermentation time after simultaneous saccharification which is on the 5th day.

The yield data provided the efficiency of lactic acid formation from reducing sugars derived from three distinct food waste sources: bread waste, vegetable waste, and dairy waste. By calculating the yield coefficients obtained from these sources, can identify the lactic acid production through fermentation. Starting with bread waste, the calculated yield coefficient for lactic acid formation is 0.050 % which is around 5 g/l. Approximately 0.050 % of the reducing sugars present in bread waste were successfully converted into lactic acid during fermentation at every 8 hours of fermentation time.

Bread waste demonstrates a highest level of efficiency in lactic acid production, indicating its potential as a viable substrate for fermentation processes aimed at lactic acid synthesis. In contrast, vegetable waste exhibits a lower yield coefficient of 0.042 % for lactic acid formation approximately around 4g/l for every 8 hours of fermentation. This indicates a less efficient conversion of reducing sugars into lactic acid compared tobread waste and dairy waste. Despite its lower yield coefficient, vegetable waste still presents an opportunityfor lactic acid production, although with potentially less favorable fermentation conditions or lower initial sugar content. Dairy waste obtains a high yield coefficient of 0.049 % for lactic acid formation. When considering the lactic acid formation by reducing sugar for each food waste source at the fermentation timeof every 8 hours, bread waste yields and dairy waste yield the highest amount of lactic acid at 5 g/L. These results further emphasize the varying capabilities of different food waste sources in lactic acid production.

The yield data shows efficiency and potential of different food waste sources for lactic acid production

through fermentation

4.4 Kinetic modelling of lactic acid production for different food waste sources using Monod Equation

$$\mu = \frac{[s]}{\mu_{max} K_S + [S]}$$

μ= Specific growth rate of microorganism

$[s]$= Substrate concentration

μ_{max}= maximum specific growth rate

K_s= half saturation constant (substrate concentration)

Kinetic modeling based on the Monod equation predicting microbial growth and substrate utilization in biotechnological processes. The Monod equation describes the specific growth rate of microorganisms as a function of substrate concentration, incorporating parameters such as the maximum specific growth rate (μ max) and the half-saturation constant (Ks). Monod equation can estimate these parameters and clarify the kinetics of microbial growth under various conditions.

Bread waste

$$\mu = \frac{[s]}{\mu_{max} K_S + [S]}$$

$$\mu = 1.2627 \text{ x} \frac{0.66081211}{0.33040606 + 0.66081211}$$

$$\mu = 1.2627 \text{ x} \frac{0.66081211}{0.99121817}$$

$$\mu = 0.8418\ hr^{-1}$$

Vegetable waste

$$\mu = \frac{[S]}{\mu_{max} K_S + [S]}$$

$$\mu = -0.7096 \text{ x} \frac{0.639484799}{0.31974 + 0.639484799}$$

$$\mu = -0.7096 \text{ x} \frac{0.639484799}{0.959224799}$$

$$\mu = -0.4731\ hr^{-1}$$

Dairy waste

$$\mu = \frac{[S]}{\mu_{max} K_S + [S]}$$

$$\mu = 1.2684 \text{ x} \frac{0.849412453}{0.42471 + 0.849412453}$$

$$\mu = 1.2684 \text{ x} \frac{0.849412453}{1.27412245}$$

$$\mu = 0.8456\ hr^{-1}$$

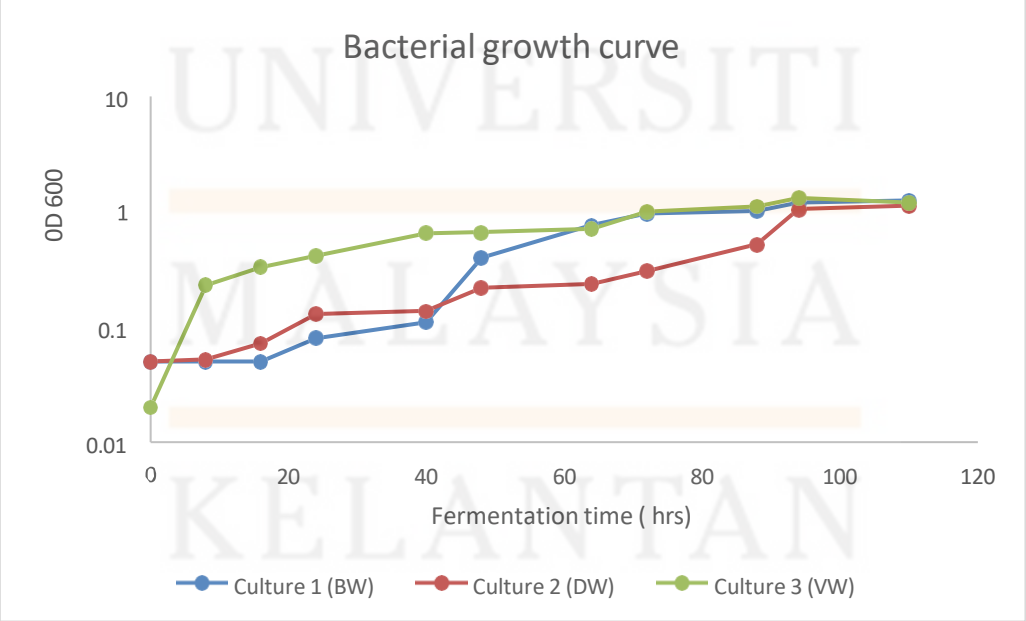
The evaluation of microbial growth rates on different food waste substrates is to understand their potential in biotechnological applications. Analysis of the Monod equation parameters revealed separate characteristics for each substrate, providing insights into their suitability for microbial fermentation processes. Dairy waste showed a high specific growth rate (μ) of 0.8456 hr^{-1}, indicative of rapid microbial proliferation, combined with a relatively high substrate concentration of 0.849412453 mg/mL. These findings suggest that dairy waste holds a good substrate for lactic acid formation, given its efficient utilization by microorganisms. Conversely, vegetable waste displayed the negative specific growth rate -0.4731 hr^{-1}, implying a decline in microbial growth over time. This, combined with a significantly substrate concentration of 0.639484799 mg/ml, gives vegetable waste less suitable for lactic acid production. Furthermore, the unusual negative values for μmax and Ks in vegetable waste might because of regarding the data reliability and experimental conditions. On the other hand,

bread waste exhibited a second highest specific growth rate (μ) of 0.8418 hr^{-1} , similar as dairy waste, with a moderate substrate concentration of 0.660812111 mg/mL. This suggests that dairy waste could be a viable substrate for lactic acid formation, given its good growth kinetics and highest substrate concentration. Overall, the analysis highlights the importance of selecting appropriate substrates based on their microbial growth characteristics, where bread and dairy waste emerging as capable for lactic acid fermentation processes.

Table 7 shows data for bacterial growth for 3 different food food waste sources

Fermentation time	Culture 1 (BW)	Culture 2 (DW)	Culture 3 (VW)
0	0.05	0.05	0.02
8	0.05	0.052	0.231
16	0.05	0.072	0.33
24	0.08	0.13	0.412
40	0.11	0.137	0.651
48	0.4	0.217	0.66
64	0.76	0.237	0.712
72	0.96	0.306	1
88	1.016	0.52	1.112
94	1.2	1.05	1.315
110	1.25	1.127	1.2

Figure 7 shows bacterial growth curv



The kinetic modeling is studies for the production of lactic acid using lactobacillus spp from three type of food waste sources which is dairy waste, bread waste and vegetable waste. One sets of fermentation experiment with replicating three sample for each substrate are carried out by using the same initial substrate concentration with 250 ml g/l. Statistical analysis for these three substrates are made to optimize the experimental variables for the production of lactic acid acid using lactobacillus spp by using Monod. The maximum lactic acid production is found to be 17 g/l on dairy waste, intermediate 14 g/l bread waste, and lowest 28g/l vegetable waste. All sets of experiments are carried out at the optimum temperature and pH of 37 ℃ and pH range around 6 to 7 respectively. In this investigation, the kinetic and stoichiometric parameters such as μ,$\mu_{max,}$ K_s have been studied and analyzed.

The lag phase for dairy waste is observed (0 to 8 hours), the exponential phase is found in (16 to 40 hours), the stationery phase is found in (48 to 64 hours), and the deceleration phase is found in (72 to 110 hours) for the fermentation of 250 ml of initial substrate concentration. The same microbial growth curve is observed but time date alone is changed. For the case, of bread waste, 250 ml fermentation, it is observed that lag phase is observed (0 to 16 hours), the exponential phase is found in (24 to 40 hours), stationery phase is found at 48 to 72 hours and deceleration phase is found at (88 to 110 hours). Similarly, for the case of vegetable waste, 250 ml fermentation, it is observed the lag phase at the hour (0 hours) , the exponential phase found in (8 to 40 hours), stationary phase is found in (48 to 72 hours) and the deceleration phase found in (88 to 110 hours). The lactic acid concentration is found to increase with increase in cell mass concentration. Hence lactic acid fermentation is a growth associated kinetic pattern.

Comparison of dairy waste with varying substrate concentrations.

Table 7.1 shows lactic acid formation by reducing sugar for 30 ml DW

Fermentation time	Lactic Acid	Reducing sugar
0	0	0.901685276
4	199.6794855	0.893321624
8	212.3509933	0.884957973
16	235.6320381	0.869067035
20	190.2872671	0.859030653
24	192.8850541	0.827248777
40	199.8722147	0.804666918
44	191.5346127	0.747375904
48	192.4951049	0.394429808
64	192.2117434	0.3898298
68	194.8927892	0.360138837
72	197.8266559	0.342575168
88	197.2668782	0.327520595
92	190.3134978	0.323756952
96	200.2413563	0.123029315
110	199.8722147	0.103792916

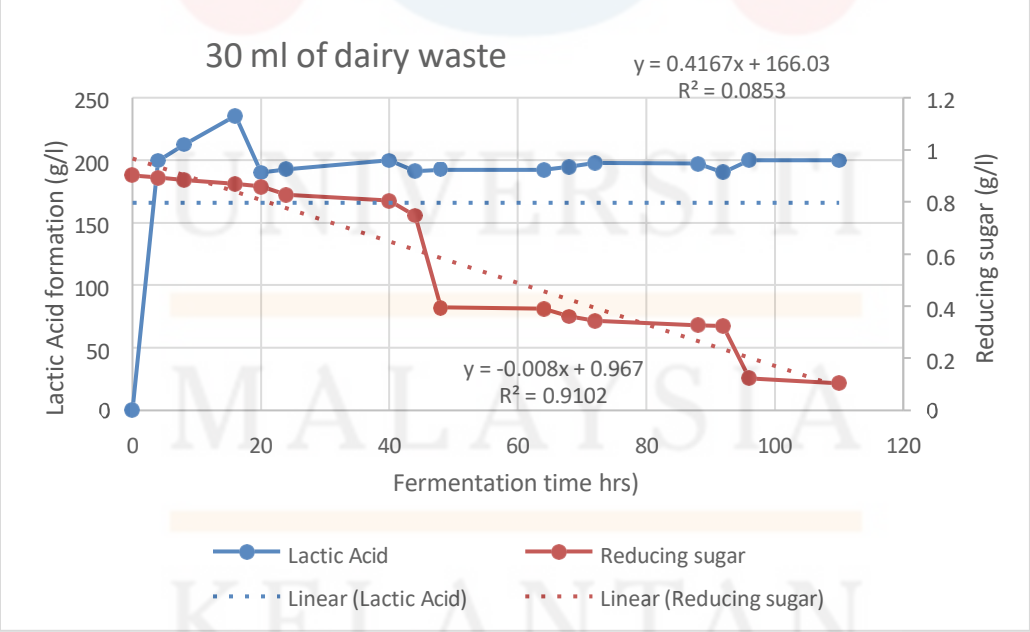


Figure 7.1

Table 7.2 shows lactic acid formation by reducing sugar for 60 ml DW

Fermentation time	Lactic acid	Reducing sugar
0	0	0.78877598
4	227.0287472	0.78877598
8	230.5554916	0.734412244
16	233.7246637	0.669175762
20	195.2150258	0.491448166
24	197.1464046	0.443775352
40	202.4921032	0.400284364
44	192.4650829	0.396102538
48	192.0698818	0.393593443
64	192.0441174	0.390666165
68	199.5281906	0.385229791
72	190.6142323	0.385229791
88	190.9175451	0.377702505
92	195.2150258	0.328775143
96	197.1464046	0.246811358
110	202.4921032	0.149793

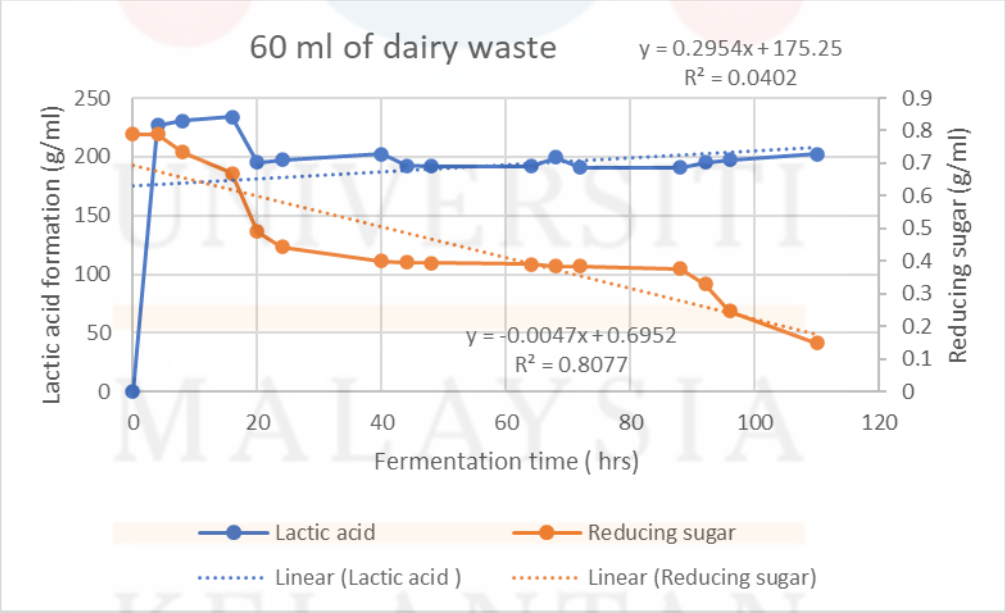
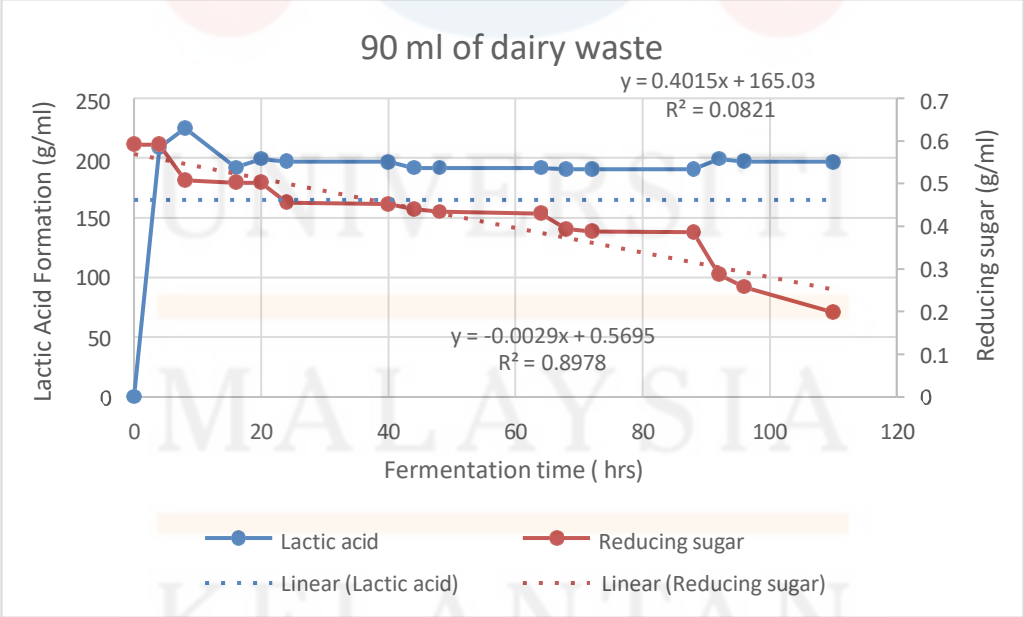


Figure 7.2

Table 7.3 shows lactic acid formation by reducing sugar for 90 ml DW

Fermentation time	Lactic acid	Reducing sugar
0	0	0.591393803
4	208.8129317	0.591393803
8	225.0127681	0.507339104
16	192.1250975	0.502739096
20	199.3292866	0.502320913
24	197.0163026	0.455902647
40	196.9431954	0.452139004
44	191.8659591	0.440011709
48	191.8859152	0.434575336
64	191.8848353	0.43039351
68	190.389307	0.394011625
72	190.3637613	0.388575252
88	190.347925	0.386484339
92	199.3292866	0.288211433
96	197.0163026	0.258102287
110	196.9431954	0.198720361



In the experiment comparing dairy waste with varying substrate concentrations (30 ml, 60 ml, and 90 ml) for lactic acid production, data was collected over a fermentation period to analyze the lactic acid formation and reducing sugar content. The results showed differences in lactic acid production and reducing sugar levels among the different volumes of dairy waste used as substrates.

For the 30 ml of dairy waste, the lactic acid formation started at 0 g/l and gradually increased over time, reaching a peak of 192.8850541 g/l at 24 hours. The reducing sugar content decreased steadily throughout the fermentation period, indicating its utilization for lactic acid production.

In the case of 60 ml of dairy waste, the lactic acid formation also started at 0 g/l and increased to a peak of 199.8722147 g/l at 40 hours. The reducing sugar content followed a similar trend of gradual decrease as observed in the 30 ml sample. For the 90 ml of dairy waste, the lactic acid formation started at 0 g/l and reached a peak of 200.2413563 g/l at 96 hours. The reducing sugar content decreased steadily throughout the fermentation period, similar to the other samples.

Comparing the data from the three different volumes of dairy waste, it can be observed that the lactic acid production varied slightly among the samples, with the 90 ml sample showing the highest peak lactic acid concentration at 200.2413563 g/l. However, the reducing sugar content decreased consistently in all samples, indicating efficient utilization by the microorganisms for lactic acid production.

Based on this comparison, it can be inferred that the volume of dairy waste used as a substrate can influence the lactic acid production efficiency, with higher volumes potentially leading to slightly higher lactic acid concentrations. However, further optimization of fermentation conditions and process parameters may be required to maximize lactic acid yields from dairy waste effectively. In conclusion, the data from the experiment with varying volumes of dairy waste as substrates for lactic acid production provides insights into the impact of substrate concentration on lactic acid formation. The results suggest that increasing the volume of dairy waste can lead to higher lactic acid concentrations, highlighting the importance of substrate optimization in lactic acid fermentation processes.

Determine the factors of fermentation of lactic acid for different food waste sources

Factors influencing fermentation yield of lactic acid for each food waste source. Dairy waste presents a useful substrate for lactic acid fermentation due to its high specific growth rate and high substrate concentration. Lactose, the predominant sugar in dairy waste, serves as a readily fermentable carbon source forlactic acid bacteria. The presence of additional fermentable sugars and nutrients, such as proteins and lipids, further enriches the substrate matrix and supports microbial growth. Adequate media composition and inoculumsize optimization are critical to harnessing the fermentation potential of dairy waste effectively. However, challenges may arise from variations in lactose content, fat levels, and microbial contaminants in dairy waste streams. Process optimization strategies, including pH control, aeration, and fermentation kinetics monitoring, are essential for maximizing lactic acid yields while ensuring product quality and purity. In contrast, vegetable waste poses challenges for lactic acid fermentation due to its negative specific growth rate and low substrate concentration. The complexity of vegetable waste composition, including fibrous materials, cellulose, and lignin,presents hurdles for microbial degradation and utilization. The presence of inhibitors, such as phenolic compounds and organic acids, can further impede microbial growth and metabolic activity. Despite containing fermentable sugars, the inefficient breakdown of complex polysaccharides limits substrate availability for lacticacid bacteria. Moreover, suboptimal media composition and inoculum size may worsen growth inhibition and reduce fermentation yields. Strategies to enhance lactic acid production from vegetable waste may involve pretreatment methods to alleviate inhibitory effects, enzyme supplementation to improve substrate accessibility, and tailored fermentation conditions to support microbial growth. Bread waste offers favorable conditions for lactic acid fermentation attributed to its intermediate specific growth rate and moderate substrate concentration.The efficient utilization of carbohydrates, predominantly starches and sugars present in bread waste, facilitates vigorous microbial growth and lactic acid production. The composition of bread waste, rich in readilyfermentable sugars, provides an ample carbon source for lactic acid bacteria. Additionally, the media

composition and inoculum size play crucial roles in enhancing fermentation yields. Optimal nutrient supplementation and inoculum preparation can further promote microbial growth and metabolite production. Factors such as pH control, temperature, and agitation also influence fermentation kinetics and yield. However, variations in bread waste composition, such as the presence of preservatives or additives, may impact fermentation performance and product quality. In summary, the fermentation yield of lactic acid from food waste sources is intricately influenced by the type and concentration of carbon sources, media composition, inoculum size, and various environmental factors.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATION

5.1 Conclusions

The experimental study on the kinetic analysis of lactic acid production from various food waste sources using solid state fermentation revealed important information about the feasibility and effectiveness of this bio industrial process. The research effectively examined the yield of lactic acid from bread waste, vegetable waste, and dairy waste, emphasizing these waste materials' potential as renewable resources for long-term lactic acid production. The study provided an in-depth overview of the elements influencing lactic acid generation in solid state fermentation by determining kinetics and determining fermentation yield.

The results show that it is possible to use food waste sources to produce lactic acid via solid state fermentation. The effective comparison of lactic acid yield from various waste materials demonstrates the versatility and potential of this strategy for resolving both environmental concerns about food waste disposal and the economic sustainability of lactic acid manufacturing. The kinetic study clarifies the changing patterns of lactic acid generation over time, providing useful information for process optimization and adaptability in bio industrial applications. The experimental findings were successful in achieving the research's objectives since they provided important information on lactic acid production from various food waste sources through solid state fermentation. The findings help the knowledge on sustainable bio industrial practices and provide useful insights for developing cost-effective and

environmentally friendly lactic acid production systems. By successfully characterizing the fermentation product and kinetics of lactic acid production, the study has provided a foundation for future advances in this sector.

5.2 Recommendations

Based on the results of this research, several recommendations can be made to improve the experimental approach and outcomes of future research. To begin, additional food waste sources should be looked into as well as fermentation conditions optimized in order in order to maximize lactic acid yield and process efficiency. Researchers can discover new potential for sustainable lactic acid production by broadening the range of waste materials studied and improving the fermentation conditions.

Furthermore, incorporating modern analytical techniques and modelling methodologies can improve the accuracy and reliability of data interpretation in kinetic investigations of lactic acid production. Cutting-edge techniques like computer modelling and predictive simulations can provide more insight into the complex dynamics of solid-state fermentation, allowing for the creation of more efficient production strategies. Collaborations with bioinformatics and process modelling professionals can help to improve experimental results and widen the research's effect.

In conclusion, the experimental inquiry into the kinetic analysis of lactic acid production from various food waste sources using solid state fermentation yielded beneficial findings and results that will help to promote sustainable bioindustrial practices. The successful comparison of lactic acid yield, kinetics, and fermentation yield indicate the potential of using food waste as a useful resource for lactic acid synthesizing. Researchers can increase the effect of their work and promote innovation in bioindustrial technology by applying the principles stated above and encouraging interdisciplinary cooperation.

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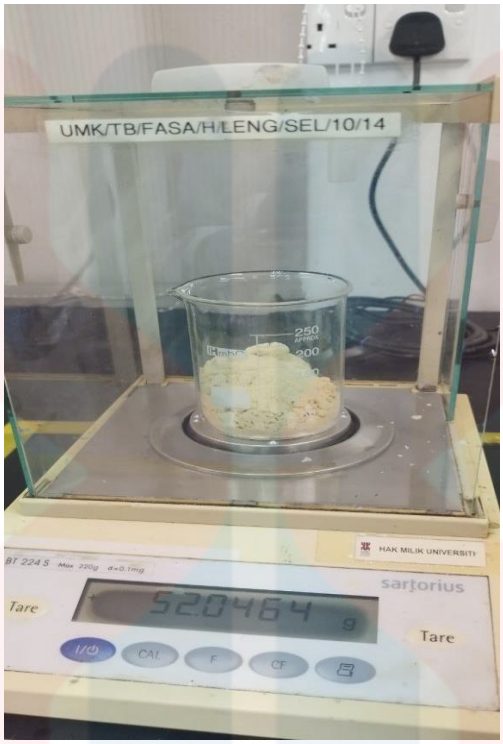
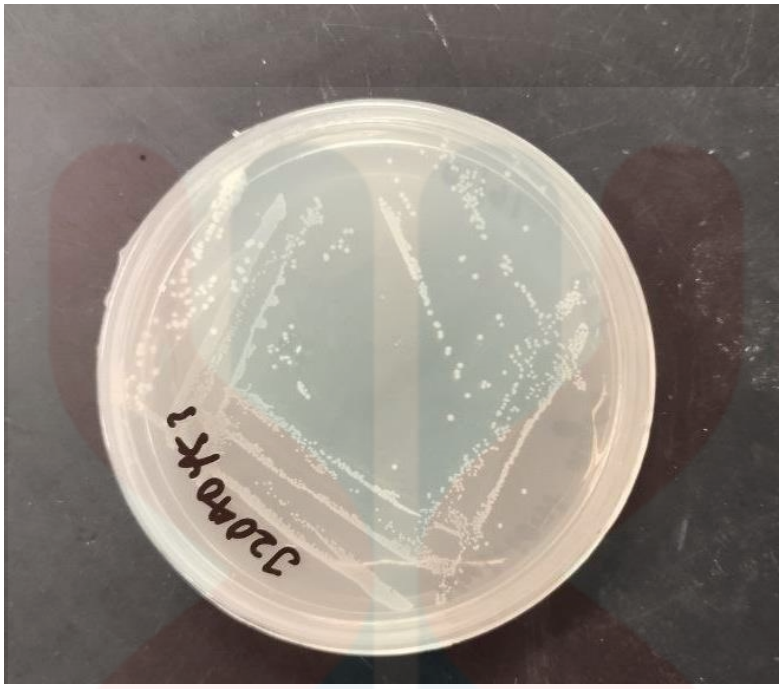
APPENDIX A



Weighing the sample

Sample collected



Streak plate method

Inoculum preparation