Investigation of Polymer Films for *E. coli* Detection

By

Praewa Thongwon 63011277

A PROJECT SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE DEGREE OF BACHELOR OF ENGINEERING IN BIOMEDICAL ENGINEERING

KING MONGKUT'S INSTITUTE OF TECHNOLOGY LADKRABANG

ACADEMIC YEAR 2023

SCHOOL OF ENGINEERING

KING MONGKUT'S INSTITUTE OF TECHNOLOGY LADKRABANG

PROJECT CERTIFICATE

Project Title	Investigation of Polymer Films for *E. coli* Detection
Student Name	Miss Praewa Thongwon Student ID.63011277
Degree	Bachelor of Engineering in Biomedical Engineering

Project Advisor Signed: _____

(Asst. Prof. Dr. Treesukon Treebupachatsakul)

Committee Signed: _____

(Assoc. Prof. Dr. Matthew Paul Gleeson)

Committee Signed: _____

(Asst. Prof. Dr. Jamie Alexander O'Reily)

Committee Signed: _____

(Asst. Prof. Dr. Pimkhuan Hannanta-an)

Committee Signed: _____

(Dr. May Phu Paing)

Head of Department Signed: _____

(Assoc. Prof. Dr. Sarinporn Visitsattapongse)

Project Title	Investigation of Polymer Films for *E. coli* Detection
Student Name	Miss Praewa Thongwon Student ID.63011277
Degree	Bachelor of Engineering in Biomedical Engineering
Project Advisor	Asst. Prof. Dr. Treesukon Treebupachatsakul
Academic Years	2023

ABSTRACT

Escherichia coli (E. coli) is gram-negative bacteria that can cause disease in humans via the consumption or handling of food products. The traditional technique of *E. coli* detection of is time consuming therefore we purpose to develop *E. coli* detection film by investigate on different polymers for *E. coli* film fabrication. The film should be able to absorb liquids from food, be resistant to liquid deconstruction, flexible and be able to emit fluorescence. The polymers used for the investigation are including Polylactic acid (PLA) and agar, which include with 4-methylumbelliferyl-β-D-glucuronide (MUG). The reaction between 4-methylumbelliferyl-β-D-glucuronide (MUG) and β-glucuronidase (GUD) which is the enzymes secreted by *E. coli*, produce 4- Methylumbelliferone (4MU) as a product that emits blue spectral fluorescence. The fabrication of PLA was conducted by (1) dissolved PLA film in solvent of ethyl acetate and (2) polymerization of 85% lactic acid by condensation under microwaving technique. Then we coated the film with 500μM MUG, 4MU, gelatin mixed with 500μM MUG, and gelatin mixed with 4MU. Then observing the result fluorescence emission of the product under a light source. For the film fabrication from agar, glycerin was used as a plasticizer and 4-methylumbelliferyl-β-D-glucuronide (MUG) was added to the solution. Then observing the fluorescence result under UV-light and measure the fluorescent intensity with captured images in MATLAB program. Moreover, tensile strength of the film was determined by tensile strength testing machine. For the results, the medium range concentration of Agar-Glycerin was the optimal biopolymer using for *E. coli*

detection film fabrication. This concentration, the Agar-Glycerin film carried out the highest fluorescent intensity, mechanical strength of Young's modulus and tensile strength.

ACKNOWLEDGEMENTS

I would like to express my deepest appreciation to my advisor Asst. Prof. Dr. Treesukon Treebupachatsakul, for her worthy guidance and support throughout my research project. Her expertise and wealth of experience were crucial in helping me to guiding through each step on developing this research project. As well as, instructing me to write a report and presentation.

I would also like to thank Asst. Prof. Dr. Kasama Srirussamee, for his assistance on providing me with the knowledges of tensile strength and mechanical properties of the film. As well as, for providing me with the resource of tensile strength testing machine. This research project will not be complete without this support.

Praewa Thongwon

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF SYMBOLS/ABBREVIATIONS

Symbols/Abbreviations	Terms
E. coli	*Escherichia coli*
PLA	Polylactic acid
GUD	β-glucuronidase
MUG	4-methylumbelliferyl-β-D-glucuronide
4MU	4-Methylumbelliferone
H_2O	Water
CO_2	Carbon dioxide
NaOH	Sodium Hydroxide
nm	nanometers
μM	micromolar
NaOH	Sodium Hydroxide
μL	microliter

CHAPTER 1

INTRODUCTION

This chapter introduces the background and information related to this project. This project purpose to investigate the appropriate polymers for film fabrication. The materials used in fabricate film include Polylactic acid (PLA) and agar. Moreover, film that is fabricated need to have an ability to detect *E. coli* in food. The research objectives are followed by hypothesis and scope of study related to this research.

1.1 Background and significance of the study

Escherichia coli (E. coli) is a bacterium which is discovered in the intestines of people and animals. *E. coli* contamination is an indicator of hygiene in water and food products. The majority of types of *E. coli* are harmless or cause relatively brief diarrhea. However, *E. coli* O157:H7 is one type of *E. coli*, which is known for producing a toxin called Shiga toxin, which can cause severe damage to the lining of the small intestine [1]. This can lead to symptoms such as abdominal pain, diarrhea (often bloody), and vomiting. Moreover, humans can receive *E. coli* through the consumption of contaminated food or water, or through contact with infected animals or their feces. Identifying and quantifying *E. coli* contamination in food and water are essential, while there are numerous techniques readily available [2]. Many traditional techniques used to detect *E. coli* such as culture-based methods and biochemical tests have high sensitivity and selectivity, but it is laborious and time-consuming [2].

The literature included in this review is a paper related to the *E. coli* detection techniques and similar processes. Treebupachatsakul et al. 2021 [2] have developed a gelatin-based microfluidic channel for quantitative *E. coli* detection. For this paper, there is some limitation which is the expense of time required to capture the video recording for intensity level. Moreover, the time required to hold the camera stationary for video recording of fluorescent product 4MU makes it is not practical without a smartphone stand. Referring to the limitation of traditional techniques used to identify *E. coli*, our research utilizes fluorescence emitted by 4-Methylumbelliferone (4MU). The 4MU is the product of the reaction between β-glucuronidase

(GUD) secreted by *E. coli*. and its substrate 4- methylumbelliferyl- β -D-glucuronide (MUG). The benefits of this project are to investigate an appropriate polymer film by including 4-methylumbelliferyl- β -D-glucuronide (MUG) substrate to measure the existence of *E. coli* by the presence of blue fluorescence [2]. The detection film is fabricated from polymer and biopolymer materials; Polylactic acid (PLA) and agar, respectively. Polylactic acid (PLA) is a biodegradable and compostable thermoplastic polymer that has high strength and water absorbable. Agar is considered as natural polymer which derived from seaweed. It is relatively inert and also biocompatible. Agar also has an ability to form gels which is useful as a solidifying agent. Therefore, I decided to fabricate the film based on both polymer and biopolymer materials.

1.2 Objectives

1.2.1 To investigate the appropriate polymer to fabricate the *E. coli* detection film which are Polylactic acid (PLA) and Agar-Glycerin.

1.2.2 To investigate the appropriate ratio of agar and glycerin for film fabrication under enzymatic reaction of 4-methylumbelliferyl- β -D-glucuronide (MUG) and β-glucuronidase (GUD).

1.2.4 To examine the mechanical property of film fabricated by agar and glycerin.

1.3 Scope of the study

1.3.1 There are 2 polymer materials including Polylactic acid (PLA), glycerin and agar used for fabricate film.

1.3.2 The detection of *E. coli* performs under the enzymatic reaction of 4-methylumbelliferyl- β -D-glucuronide (MUG) and β-glucuronidase (GUD).

1.4 Report outline

The contents provided in this report is organized as follows:

Chapter 2 reviews the theory related in this comprehensive study

Chapter 3 describes the design and methodology involved film fabrication

Chapter 4 indicates the result and discussion

Chapter 5 reviews both result and discussion as well as conclude the key result related to this research, along with suggestion for the future work.

CHAPTER 2

THEORY

This chapter provide the discussion of theory related to this comprehensive study, background and design. In the section 2.1 clarify the polymer used for film fabrication. Plasticizer used for film fabrication is mentioned in the section 2.2. Section 2.3, explain the enzymatic reaction between 4-methylumbelliferyl-β-D-glucuronide (MUG) and β-glucuronidase (GUD) secreted by *E. coli.* Lastly, the section 2.4 is the measurement tensile strength of the film.

2.1 Polymer used for film fabrication

2.1.1 Polylactic acid (PLA)

Polylactic acid (PLA) is a polymer of lactic acid as shown in figure 1, derived from renewable resources, such as wheat and straw, which are completely biodegradable and compostable thermoplastics. The mechanical properties of PLA vary from soft and elastic to stiff and hard materials. It is considered as a stiff and brittle material; however, the speed and crystallinity rate of PLA can be controlled [3]. PLA has a relatively low melting point temperature and high glass transition temperature compared to other thermoplastics [3], as shown in the table 1. The glass transition temperature can affect the texture of PLA. PLA becomes softer when heated through its glass transition temperature.

Table 1. Melting point and Glass transition temperature of different polymers [4]

Polymer	Melting Point Temperature	Glass Transition Temperature
Polylactic acid	170 ℃	55 ℃
Polypropylene	75 ℃	-10 ℃
Polyglycolide	220-225 ℃	35-40 ℃

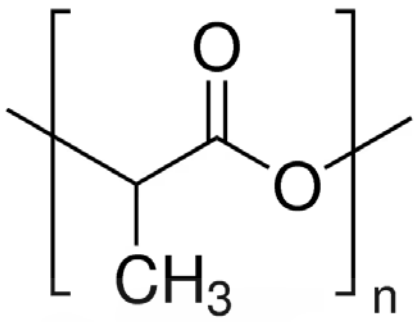


Figure 1. Chemical structure of Polylactic acid [23]

PLA is mostly used in the food industry to package sensitive food products. The permeability performance of food packaging against the transfer of substances and aroma is important, as PLA has high performance in capturing aroma and providing a low barrier towards water vapor and oxygen [3]. PLA also shows better water permeability compared to oriented polystyrene.

The biodegradation process of PLA occurs naturally in the presence of bacteria and algae. During this process, PLA is broken down into H_2O, CO_2, and other inorganic compounds. To ensure the biodegradability of PLA, specific conditions including pressure, humidity, and temperature need to be monitored. This is because these conditions have a significant effect on speeding up or slowing down the rate of biodegradation [4]. There are two phases of biodegradation, which are heterogeneous and homogeneous. Heterogeneous degradation takes place on the surface of the polymer, while homogeneous degradation occurs intramolecularly. Moreover, various factors such as melting point temperature, glass transition temperature, molecular weight, chemical affinity, and many more influence biodegradation. For example, the rate of biodegradation will be slower if the melting point is high.

In this comprehensive study, we have employed a PLA base material as a fundamental building block to synthesize the thin film under investigation. As we mentioned about the properties of PLA, the properties of PLA were mainly influenced by the structure of it. Emphasizing the structure of PLA, as shown in figure 1, the properties of PLA can vary based on structural factors, including component isomer, molecular weight, annealing time, and temperature. Therefore, the crystallinity of PLA can be affected by stereochemistry and thermal

history. By influencing its crystallinity, other properties, such as hardness, tensile strength, stiffness, melting point, and modulus, may also be impacted.

Increasing the molecular weight number can reduce the crystallinity of PLA; however, it might also increase tensile strength and shear viscosity. The properties, including tensile strength and shear viscosity, depend on the polymer chains in the chemical structure of PLA. The chemical structure of PLA results in different forms depending on stereochemistry variables, which can lead to either semi-crystalline or amorphous forms in PLA [5]. Moreover, considering the stereochemistry variables, the ratio of D-content and L-content of PLA as shown in figure 2 is also important. The D-content is an important parameter that enables the alteration of PLA's properties in several ways. For instance, increasing the D-content in PLA can lead to a lower rate of crystallization [5]. As the rate of crystallization decreases, it results in a lower melting point too.

To improve the crystallinity properties of PLA, it can be blended with other polymers such as starch or polyethylene glycol.

Figure 2. D-content and L-content in Polylactic acid [6]

Looking more into the stereoisomers of PLA, it consists of various stereoisomers such as poly(L-lactide) (PLLA), poly(D-lactide) (PDLA), and poly (DL-lactide) (PDLLA). PLA is one type of linear aliphatic thermoplastic polyester that belongs to poly-α-hydroxy acid. Therefore, D and L content are factors that affect the enantiomeric form of PLA. The enantiomeric form of PLA can be separated into three different categories, including levorotatory (L-), dextrorotatory (D-), and meso(a combination of L- and D-) [6] as shown in the figure 3. These categories of PLA forms depend on the source and process used to produce it. The main fraction of PLA coming from biological sources is the L-isomer, which is considered a biological metabolite. Therefore, PLLA has long degradation times along with high crystallinity. To reduce

crystalline formation, PLLA needs to be combined with a polymer or other material that consists of L-lactic and D-lactic or L-lactic monomers.

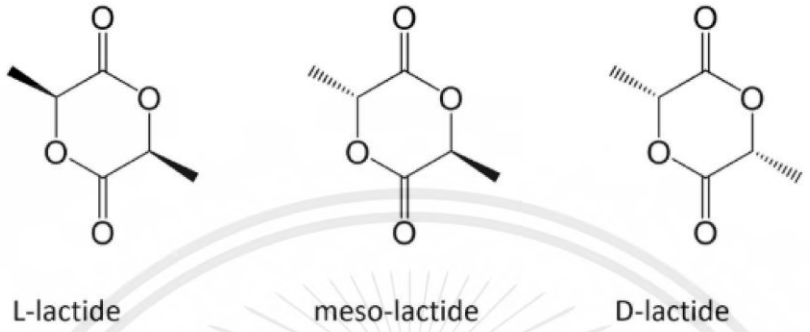


Figure 3.3 categories of enantiomeric form [24]

- **Applications of PLA**

1. 3D printing material filament

The 3D printing filament is an affordable and useable plastic. The material should be easily used for prototypes and pattern making. As PLA produced from renewable resources, it was become a widely used material due to its environmentally friendly material. PLA, which comes in the form of wire, is easy to feed into the extruder head, where it melts the PLA and extrudes it onto the printing tray [7]. Moreover, PLA shrinks less than Acrylonitrile butadiene styrene (ABS) when cooling, which provides good stability during the manufacturing process.

Figure 4. 3D printer filament [25]

2. Medical implants

Referring to the properties of PLA showing that it is biocompatible and biodegradable, PLA has become widely used as a biodegradable polymer in medical applications. Once the polymer is exposed to biological media, it will break down and can be excreted through urine and breath [4].

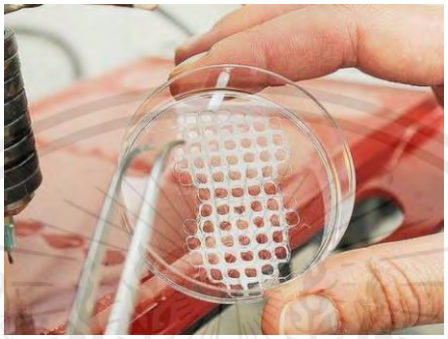


Figure 5. Medical implants [26]

3. Packaging industry

In the packaging industry, PLA is often used to produce plastic films and food containers. As PLA is made from renewable resources and has various properties, it is suitable to be used with food. Moreover, PLA is a natural polymer that is produced to substitute the use of petroleum-based plastics such as polyethylene terephthalate (PET) [3].

Figure 6. Compostable PLA biodegradable film [27]

2.1.2 Agar

Agar is a natural polysaccharide derived from seaweed, especially red algae. Agar is composed of agarose, which functions as the gel-forming component. Another composition of agar is agaropectin that shown in figure 7, which is a branched, non-gelling component [8]. The texture of agar comes in the form of a white powder a shown in figure 8 and soluble in hot water.

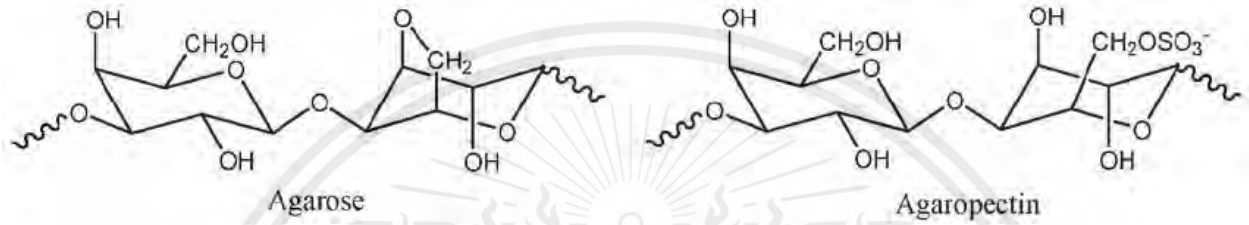


Figure 7. Chemical structure of Agarose and Agaropectin [28]

Figure 8. Agar powder [29]

Agarose is composed of repeating units that are between 3-linked β-D-galactopyranosyl (G) and 4-linked 3,6-anhydro-α-L-galactopyranosyl (LA) units. Agaropectin is composed of a sulfated polysaccharide (3% to 10% sulfate), ester sulfate, D-glucuronic acid, and some small amounts of pyruvic acid. The ratio between agarose and agaropectin varies depending on the species of seaweed. To achieve agar gel, the gelling portion should have a double helical structure. The helix is formed by three equatorial hydrogen atoms on the 3,6-anhydro-L-galactose residues [9]. Then, the double helix structure will form a three-dimensional framework that holds the water molecules inside the framework.

Agar is widely used in the food industry, pharmaceuticals, cosmetics, and is also used as a gelling agent in microbiological culture. Due to its high gelling strength, agar is used to solidify culture media. The melting temperature of agar is 90-95 ℃, and it has a setting temperature of 32-39 ℃. Regarding its gelling power, agar gels can also be produced in very dilute solutions, ranging from 0.5% to 1.0% of agar. However, most agars used in bacteriological work create firm gels at a 1.5% concentration, which do not melt if the temperature does not exceed 85 ℃ [10]. The strength of the gel created from agar can be influenced by various factors such as the concentration of agar used, time, pH, and also sugar content. For instance, if the pH is too low, the gel strength will be weakened as well. The wide range of gel-forming and melting points provides agar with unique and useful properties. The significant difference in gel-forming is an important factor that can be applied for various applications, such as creating gels or films with varying hardness. Moreover, agar is also considered a non-toxic material that can be applied in different uses.

Even though agar does not dissolve in cold water, it swells considerably and absorbs as much as its own weight of water. Agar also dissolves in a variety of solvents at room temperature. Furthermore, the viscosity of agar also varies widely depending on the raw material source. The viscosity of agar gel will start to become relatively constant at pH 4.5 to 9.0 after the temperature exceeds its melting point. As the viscosity of agar can vary, adding different plasticizers can enhance the viscosity rate of agar as well. The agar gels are quite stable, but their stability depends on two factors, which are electric charge and hydration. If these two factors were removed, there would be flocculation of agar occurring in the solution. Moreover, lengthening the time of agar gels at high temperatures can degrade the solution and lower gel strength after the gel is well formed. For that reason, agar solutions should be avoided from exposure to high temperatures for long periods of time. Even though the dry solution is not prone to contamination by bacteria or microorganisms, agar solutions and gels are suitable fertile media for bacteria.

- **Application**

1. Food industry

As agar solution is well soluble in hot water and swells considerably in various solvents too, the different properties of agar make the gel very useful as an additive to use in the food industry. Agar can be used as an additive with various products: milk, confectionery, beverages, and bakery items. Here are some examples of products: ice cream, yogurts, candy bars, refining of juices, sugar icing, and many more [8].

Figure 9. Agar dessert [30]

2. Medical and Pharmaceutical Industry

Agar gel has the property of inhibiting the liquefaction that occurs due to enzyme action of microorganisms. This allows for a variety of applications in the medical and pharmaceutical industries [11]. For instance, agar is used as a fertile medium for preparing bacterial cultures in microbiology, a retarding agent and carrier in medicines, and also used as a stabilizer in cholesterol solutions.

3. Biotechnology

In the field of plant biotechnology, agar has long been used as a solidifying medium for plant tissue culture. Referring to the gelling properties of agar, it is one factor that is important to the production operation. They help support the growth of cultures and the development of roots.

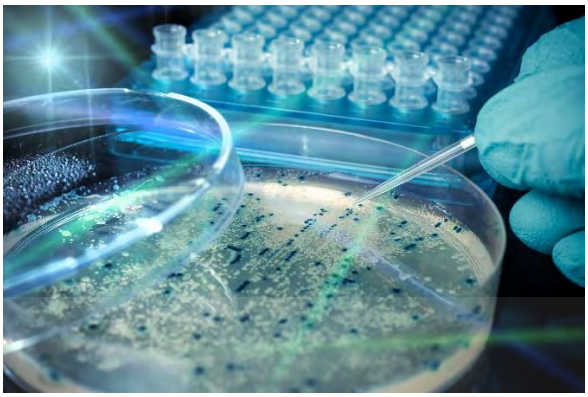


Figure 10. Culture media [31]

2.1.3 Gelatin

Gelatin is considered a natural protein derived from raw collagen. As gelatin is made from a natural polymer, it is usually produced from pig skins and beef bones because they have a high concentration of raw collagen. It is a solid and translucent substance that is neutral in color, taste, and odor. The production of gelatin also helps prevent food wastage. It is ensured that gelatin is a safe and well-regulated material, as the manufacturing process of gelatin undergoes strict testing to control the quality and safety [12].

Figure 11. Gelatin powder [32]

As the gelatin is extracted from collagen and is composed of the repetition of Gly-X-Y as shown in figure 12 and 13 which are glycine, proline, and hydroxyproline respectively [13]. The structure

of gelatin consists of cationic groups, anionic groups, and hydrophobic groups in approximately the same ratio. Expanding further on each group, within the polypeptide chains, there are approximately 13% of positively charged amino acid residues, 12% of negatively charged amino acid residues, and around 11% of hydrophobic residues.

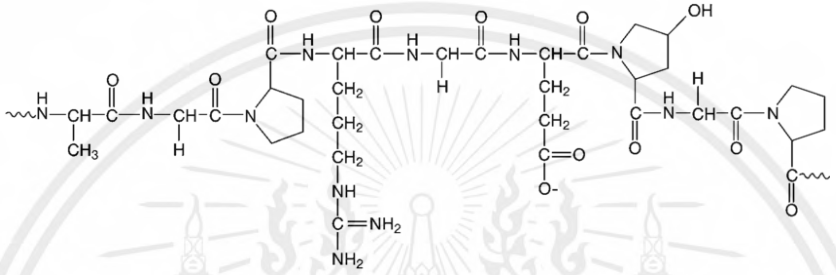


Figure 12. Chemical structure of gelatin [33]

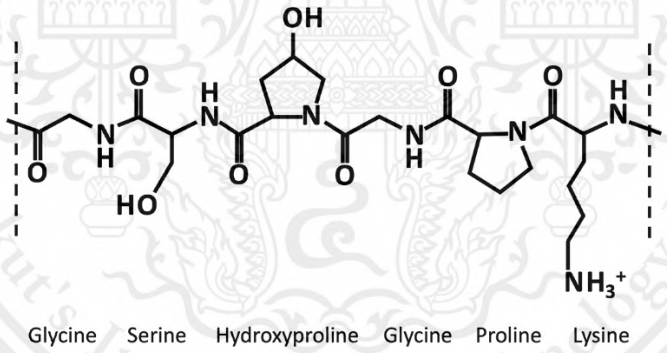


Figure 13. Chemical structure of a fragment of gelatin [34]

Gelatin has a melting point at 31.7 - 34.2 ℃, which means it melts around body temperature. As gelatin comes from a natural product, which makes it is a perfect clean label ingredient. Gelatin is a pure protein, so it is non-allergic, cholesterol-free, and fat-free. Moreover, gelatin has unique properties of thermo-reversibility [14]. The solution containing gelatin concentration becomes a gel when cooled down and returns to a liquid state when exposed to heat for a long period of time. This reversible transformation can be repeated and does not show

significant changes in characteristics. As mentioned, gelatin comes in the form of a solid and translucent material, so when gelatin is hydrated, it produces a flavorless and flexible film or transparent gel.

- **Applications**

1. Pharmaceutical Field

In this field, gelatin gives various applications, for example, the production of capsules or tablets, components of wound dressing, and blood volume substitutes. Due to its unique adhesive, gelling, and film-creating properties [15], gelatin is an important excipient for the manufacturing process of capsules. Using gelatin-containing capsules, the medicine or supplement is well-preserved and given a long shelf life as well.

Figure 14. Gelatin capsules [35]

2. Food industry

In the food industry, gelatin has been used for different purposes. Gelatin has been widely used as a development component of edible films and coatings. The concept of edible films and coatings stems from environmental issues. Edible coatings usually come in a liquid form for dipping or spraying on fruits or vegetables. As gelatin is extracted from collagen, it has biodegradability, good antibacterial, and antioxidant properties.

Figure 15. Edible coating in fruit [36]

2.2 Plasticizers used for film fabrication

2.2.1 Glycerin

Glycerin, or glycerol, is a mixture of sugar and alcohol, resulting in an organic alcohol. It is colorless, odorless, and non-toxic to nature. Glycerin has a chemical formula that contains three hydroxyl groups as show in figure 16, so it is soluble in water. Due to its properties, glycerin is widely used in various manufacturing processes such as pharmaceuticals and as a sweetening agent in different applications. The molecular weight of glycerin is normally around 92.09 g/mol, with a boiling point of 290 ℃ and a melting point of 17.8 ℃ [16]. Under standard conditions of both temperature and pressure, glycerin forms miscible mixtures with water.

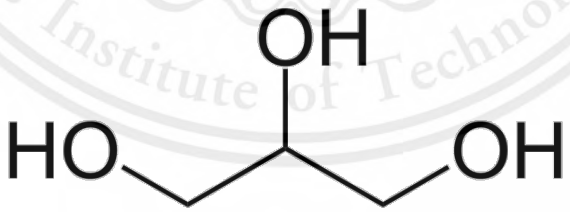


Figure 16. Chemical formula of Glycerin [37]

Glycerin can be manufactured from different materials: plant-based materials, animal-based materials, and synthetic materials. Glycerin that is derived from plant-based materials is considered natural glycerin and is obtained from oils and fats of coconut and palm.

Because it is an organic material, its texture is similar to syrup, and it is also vegan. Glycerin made from animal-based materials is derived from fat. During the manufacturing process, the hardened mixture needs to be boiled to remove excess water and impurities. Lastly, glycerin produced from synthetic materials is made from petroleum-based sources and cane or corn syrup as well.

2.3 Enzymatic reaction

In *Escherichia coli*, also known as *E. coli*, it contains an enzyme called β-glucuronidase. This enzyme is responsible for the hydrolysis of glucuronide compounds and has the ability to serve various purposes, including the metabolism of sugars, detoxification, and as a research tool [17]. Sugars can be used as carbon sources for the growth of *E. coli*, and β-glucuronidase functions in the metabolism of glucuronide sugars. Moreover, this enzyme can also detoxify harmful compounds. If toxic substances enter the cell, they will bind with glucuronic acid, resulting in glucuronide compounds. When glucuronides occur within a cell, β-glucuronidase can hydrolyze these compounds to release the toxic substances and expel those substances out of the cell. Lastly, β-glucuronidase is widely used in molecular biology and microbiology research, mostly as a reporter gene [17].

4-Methylumbelliferyl-β-D-glucuronide, or MUG, is a fluorescent substrate of β-glucuronidase. MUG is a synthetic substrate commonly used to assess the presence and activity of glucuronidase enzymes. As MUG is a synthetic substrate, the appearance of it is crystalline powder; therefore, the physical state of it is solid. Due to its appearance, MUG powder needs to be stored in the freezer, and the container needs to be closed tightly, then kept in a dry and well-ventilated place. Moreover, materials that are known as strong oxidizing agents, strong acids, and strong bases are incompatible materials with MUG.

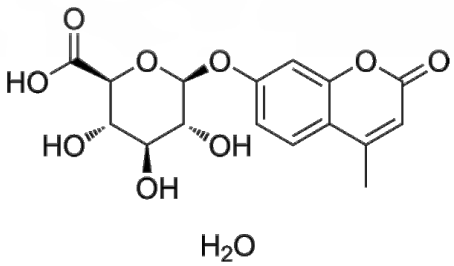


Figure 17. 4-Methylumbelliferyl-β-D-glucuronide (MUG) chemical formula [38]

This reaction occurs when the glucuronidase enzyme is active. Then, the 4-Methylumbelliferyl-β-D-glucuronide is cleaved by β-glucuronidase, resulting in the release of the blue fluorescence of 4-methylumbelliferone (4MU) as the reaction shown in figure 18. 4-methylumbelliferone (4MU) is the product resulting from the reaction between GUD and MUG, and the blue fluorescence emitted by 4MU can be observed under UV light [2].

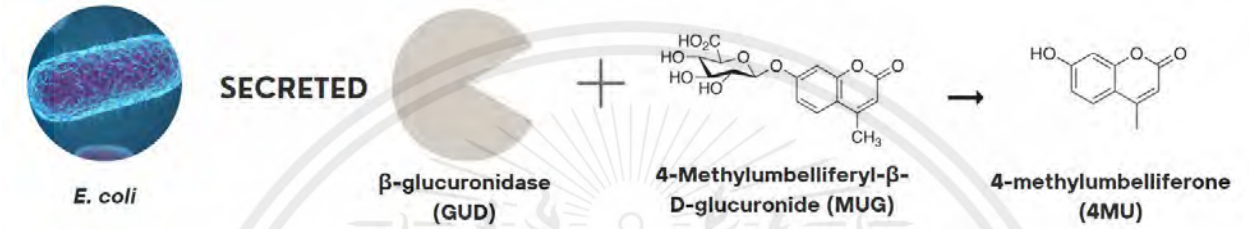


Figure 18. Enzymatic reaction of GUD and MUG

4MU is a fluorescent compound that emits light in the blue spectral range at 450 nm and when excited by light is around 355 nm as shown in figure 19. The detection of 4MU fluorescence product can be quantified using different techniques such as fluorescence plate readers or other fluorescence-based techniques. Moreover, the intensity of the measurable fluorescence (4MU) is directly proportional to the amount of β-glucuronidase.

Figure 19. 4MU fluorescence under the UV-light

2.4 Tensile strength of the film

The ability of material to withstand the force before it breaks is consider as a tensile strength of the material. Tensile strength is used to measure the maximum tensile stress of a material [18]. The tensile strength can be measure by using the tensile strength testing machine to determine the load strength. The basic principle of this machine is applying force to the material in an axial load until those material break or fracture. Tensile strength is an essential factor using in designing factors such as building or bridges. It is also use with the product which need to withstand the force from pulling and stretching as well. It can be used to evaluate the material is suitable for its application or not. For instant, tensile strength of material in the aerospace industry is used to determine the safety of the aircraft [18].

There are various factors which affect the value of tensile strength in materials. Different materials consist of different molecular structure. The molecular structure provides an intermolecular force inside the material which helpful in binding the molecule. By changing the molecular of the structure can mainly affect the tensile strength of materials. Not only molecular structure that affect the tensile strength of material, temperature also one of these factors as well [18]. Molecular structure of material is corresponded to the temperature, if the temperature of the material increased, it will lead to enhance the bond strength of molecules. However, if the temperature is low, in contrast the material might lose some of its bond strength or tensile strength.

Tensile strength relates to Young's modulus. It is directly proportional to each other. Young's modulus is used to determine the elasticity of a material. This indicates that if the tensile strength increase, Young's modulus will also increase too. As a material which can withstand high Young's modulus, the material become stiffer and hard to be deform. Tensile strength provides the data of load apply on the material and the displacement when the material was pulling by the machine. This data can be used to calculate Young's modulus as shown in the equation in the next section [19].

This research uses tensile strength and Young's modulus to determine the mechanical properties of *E. coli* detection film fabrication. Tensile strength and Young's modulus was used to identify the ability of film deformation.

Equations used to calculate Young's modulus from data of tensile strength are shown as following [17]:

$$\sigma = \frac{P}{A_0} \qquad \text{(stress)}$$

$$\varepsilon = \frac{L_f - L_0}{L_0} = \frac{\Delta L}{L_0} \qquad \text{(strain)}$$

$$E = \frac{\sigma}{\varepsilon} \qquad \text{(Young's modulus)}$$

σ = stress

ε = strain

P = external tensile load

A_0 = original cross-sectional area

L_0 = original length

L_f = final length

CHAPTER 3

METHODOLOGY

In this chapter, the preparation of Polylactic acid solution and agar solution for film fabrication is mentioned. Moreover, the information about biomarker *E. coli* detection and mechanical properties of the film are identify as well.

3.1 Film fabrication

3.1.1 Preparation of Polylactic acid (PLA) and gelatin film

Polylactic acid and gelatin are considered polymer, which can be used to make polymer-based products, including films. The PLA used in this case is commercial PLA in the form of strings. To facilitate its dissolution, the PLA needs to be cut into small pieces. Ethyl acetate is used as a solvent to dissolve the PLA. The ratio of PLA to ethyl acetate is 5 g and 45 mL, respectively. It is heated to 120℃ with stirring until the solution becomes completely homogeneous, as shown in figure 20. Afterward, pour the solution into glass petri dishes and glass plates to achieved a thin film as shown in figure 21. The mixture of PLA and ethyl acetate needs to be poured onto the glass surface because the ethyl acetate solution can dissolved the plastic petri dishes.

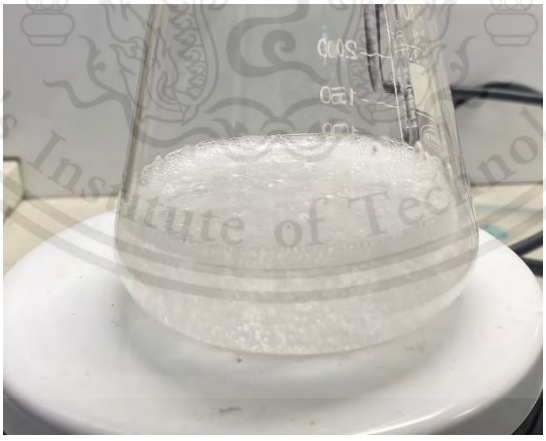


Figure 20. PLA and ethyl acetate solution

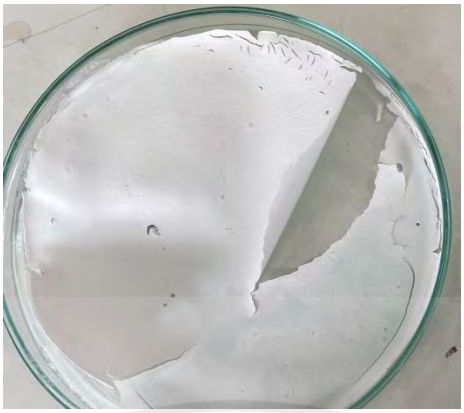


Figure 21. Solution pours into glass petri dishes

After the product has dried, cut it into small pieces and then immerse the particles in ethyl acetate for 15 minutes to make the film's surface sticky. Next, coating 500μM 4MU and 500 μM MUG, and gelatin with 4MU and MUG at the same concentration on the film by immersing film in the solutions. Then allow the film to settle in the solution for 5 minutes. The purpose of coating the PLA film with gelatin is to increase the films absorbable. The ratio of gelatin to distilled water is 2 g and 6 mL, respectively.

3.1.2 Preparation of Polylactic acid (PLA) synthesis

Another technique used to synthesize PLA involves microwaving lactic acid at a high-power level. The material is prepared by microwaving 20 mL of 85% lactic acid at 950W for 9 minutes. During this process, water is removed lead to the condensation of the lactic acid. Furthermore, this procedure effectively increases the molecular weight of the PLA. To raise the pH of the PLA, 60 drops of sodium hydroxide (NaOH) were added, resulting in the pH was change from 4 to 6. Afterward, pour the prepared solution onto filter paper and place it in a 90 ℃ oven for 4 hours and 30 minutes to dry and solidify the sample as shown in the figure 22.

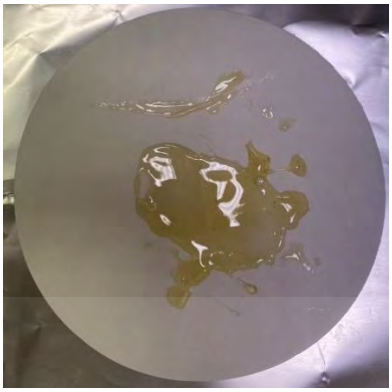


Figure 22. Polylactic acid synthesis

3.1.3 Preparation of agar and glycerin

For this research, the ratio of agar and glycerin is separated into three different ranges: high range, medium range and low range as shown in the table 2, 3 and 4 according to the study of V. Ibarra, 2022 [20]. The mixture of agar, glycerin, and distilled water is prepared by stirring and heating at 135 ℃ on the hot plate for 10 to 15 minutes until the solution becomes homogeneous. Then, pour the solution onto the plastic petri dishes and let it settle for 3 days. After the agar and glycerin solution dries up, the medium-range solution shows the most appropriate film for this experiment, as shown in the figure 23. The medium range solution was also reduced to 20 mL to ensure that one portion of the solution fits perfectly into the plastic petri dishes. To prevent contamination during the process, another technique used to prepare the film is to place the mixture solution inside an oven and turn on the heat to 40 ℃, allowing the solution to settle inside the oven for 2 days, this technique can prevent the contamination. Moreover, after we decided to used the medium range solution to fabricated the film, the solution of MUG is added as shown in the table 5 for further experiment.

Table 2. 100 mL of High range solution concentration [20]

Condition	Agar	Glycerin	Distilled water
1	8.00 g	5.35 mL	94.65 mL
2	4.00 g	10.70 mL	89.30 mL
3	8.00 g	10.70 mL	89.30 mL

Table 3. 100 mL of Medium range solution concentration [20]

Condition	Agar	Glycerin	Distilled water
1	3.00 g	1.25 mL	98.75 mL
2	2.00 g	1.88 mL	98.12 mL
3	3.00 g	1.88 mL	98.12 mL

Table 4. 100 mL of Low range solution concentration [20]

Condition	Agar	Glycerin	Distilled water
1	1.00 g	0.31 mL	98.69 mL
2	0.50 g	0.63 mL	98.87 mL
3	1.00 g	0.63 mL	98.37 mL

Table 5. 20 mL of Medium range solution concentration

Condition	Agar	Glycerin	MUG	Distilled water
1	0.6 g	0.25 mL	250μM, 10 mL	9.75 mL
2	0.4 g	0.376 mL	250μM, 10 mL	9.624 mL
3	0.6 g	0.376 mL	250μM, 10 mL	9.624 mL

Figure 23. Thin film achieved from medium range solution

3.2 Biomarker *E. coli* detection

3.2.1 Biomarker *E. coli* detection for Polylactic acid and Polylactic acid synthesis film

For the preparation of MUG solution, the concentration of MUG initially prepared at 500 μM in 0.05 M phosphate buffer. The preparation of GUD solution is similar to the MUG solution, as we also used the diluted phosphate solution. So, 99.99 mL of the diluted phosphate solution is mixed with 10 L of GUD solution. The solution obtained is 100 mL of 1700 GUD.

During the process of detecting the *E. coli* biomarker GUD in the PLA film and PLA synthesis film, they were separated into four categories: coating the film with 500μM MUG, 4MU (control), gelatin mixed with 500μM MUG, and gelatin mixed with 4MU (control). After soaking the film in ethyl acetate for 15 minutes, the surface of the film became sticky. The films were separated for each category and soaked in each solution for another 15 minutes. Then, the solution of GUD was dropped on top of the film, allowing the film to absorb the solution for 5 minutes, and fluorescence was observed under UV light as shown in the figure 24 and 25.

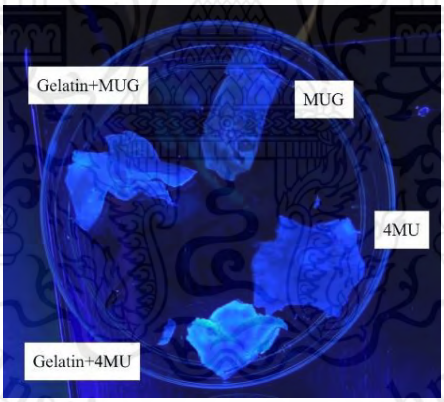


Figure 24. Fluorescence emitted from PLA film in each category observed under UV-light

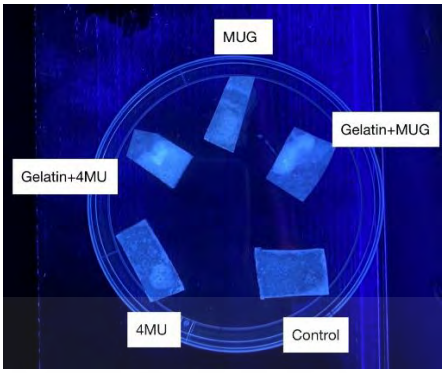


Figure 25. Fluorescence emitted from PLA synthesis film in each category observed under UV-light

The next step is to confirm whether the MUG solution has leaked out and is causing the reaction outside of the film. After observing the film under UV light, we removed the excess solution from the top of the film and examined it under UV light. If fluorescence is present under the UV light, this indicates that there is MUG leaked out from the film and MUG-GUD reaction has occurred outside of the film as shown in the figure 26.

Figure 26. Solution of MUG leaked from the film

3.2.2 Biomarker *E. coli* detection for Agar film

For the process of detecting the biomarker *E. coli* in agar film, the MUG solution is already mixed with the agar film solution. After the agar film has dried up, 100 μl of GUD is dropped on top of the film, and wait for the reaction to occur for 10 minutes. Afterward, the film is observed for fluorescent color under the UV-light.

The preparation of the film to test the reaction involved cutting the film into 8 pieces for different conditions. According to table 3-5 To measure the fluorescent intensity, the film is separated into 8 pieces: 4 for the control group, where MUG is not added to the agar solution, and the other 4 pieces are for observing fluorescence resulting from the enzymatic reaction of MUG and GUD at reaction times of 5 minutes, 10 minutes, 20 minutes, and 30 minutes. The intensity of the resulting fluorescence is measured using the MATLAB program with the images captured. The images used for analyzing fluorescent intensity are captured from both the solution of MUG leaked from the film and the film that has been removed excess solution. Since 4MU emits blue fluorescence, the code is designed to eliminate other colors and analyze only the blue color emitted from the film as shown in the figure 27.

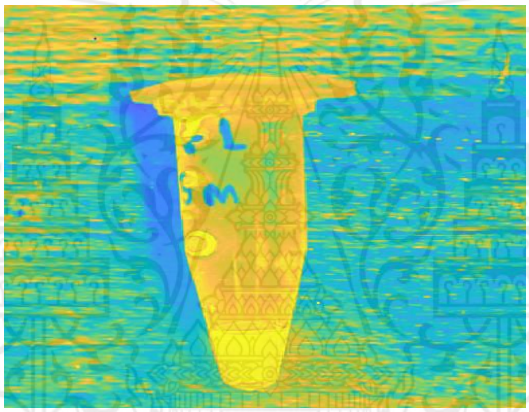


Figure 27. Image after analyze the blue color emitted from MUG-GUD reaction

3.3 Process of testing agar film mechanical properties

The machine used to test the mechanical properties of the film is the machine which can measure the tensile strength. For the film preparation, the film needs to be cut into a bone shape as shown in the figure 28 so the machine can grip the top and bottom part of the film. The working fundamental of this machine is to pull the specimen according to the set-up speed. To measure the tensile strength of the film, set-up speed is 2 mm per minute [22]. After setting up the machine, the film needs to be manually stretched until the load appears on the screen, and then start running the machine. Next, wait until the film is torn apart from each other and collect the data for further calculation. Repeating this step for each specimen from all conditions, all of the data will be used to calculate the modulus of different conditions

In this procedure, stress and strain are used to calculate the value of Young's modulus. Stress can be calculated by using the data of external tensile load divided by the value of the original cross-sectional area of the specimen. Each condition has the same value of original cross-sectional area but different external tensile load depending on the amount of agar and glycerin. For the value of strain, this can be identified by using the original length of the specimen divided by the final length after it has been pulled apart. By calculating these values, amount of Young's modulus can be defined by using stress divided by strain.

It can be represented by the equation shown following:

$$\sigma = \frac{P}{A_0} \qquad \text{(stress)}$$

$$\varepsilon = \frac{L_f - L_0}{L_0} = \frac{\Delta L}{L_0} \qquad \text{(strain)}$$

$$E = \frac{\sigma}{\varepsilon} \qquad \text{(Young's modulus)}$$

σ = stress

ε = strain

P = external tensile load

A_0 = original cross-sectional area

L_0 = original length

L_f = final length

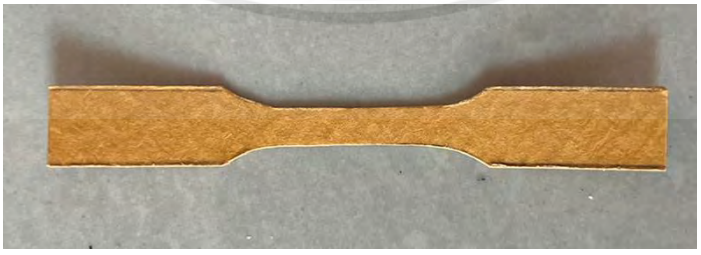


Figure 28. Bone shape model for film mechanical test

CHAPTER 4

EXPERIMENTAL RESULT AND DISCUSSION

In this chapter, the results of film fabricated from Polylactic acid (PLA) in ethyl acetate soluble, Polylactic acid (PLA) synthesis and agar with glycerin as a plasticizer are presented. The film fabrication parameters, such as time, concentration of each solution and containers used for stored fabricating film were examined. Moreover, the results of the GUD-MUG reaction, fluorescent intensity and tensile strength are also mentioned in this section as well. Additionally, the fluorescent intensity from GUD-MUG reaction is measured with different reaction time period.

4.1 Polylactic acid (PLA)

4.1.1 The result of film fabrication from Polylactic acid (PLA)

The sample of PLA dissolved in ethyl acetate solution was a white powdery solid. According to its properties, PLA undergoes heated at the temperatures above its glass transition temperatures which can increase the strength of PLA. However, the sample might also become brittle as well. On the contrary, some of the results turn out to be transparent film which requires the solution to be cooled down rapidly and the layer of the film should be thin enough.

To examine the thin film fabrication of PLA dissolved in ethyl acetate, dissolved PLA solution was pouring on different containers including glass plate, beaker, glass petri dish, plastic petri dish and aluminum foil. Different containers produced different textures and thickness of the film. The table 6, shows the result of film fabrication from PLA by pouring on different containers. It was found that different containers affect the texture and thickness of the PLA film. Pouring on the glass plate, the texture of the film resulted in thin layer because the glass plate pass through the water at room temperature before pouring the dissolved PLA on the glass plate. Moreover, the sample could be easily peeled off with rinsing of the water. For the beaker, the dissolved PLA solution was pouring on the bottom part of the beaker resulted in thin layer white powdery solid. The glass petri dish also has similar texture of film as the beaker. However, the sample produced from the glass petri dish gave out slightly thicker layer as the glass petri dish has a smaller surface to poring the PLA solution. For the plastic petri dish, this container could not be

used because the ethyl acetate solution can dissolve the plastic as well. Lastly, the sample produced from the aluminum foil came out as a brittle film. In summary, glass plate and glass petri dish are suitable container for fabricating the PLA film.

Table 6. Various containers used for film fabrication from Polylactic acid (PLA)

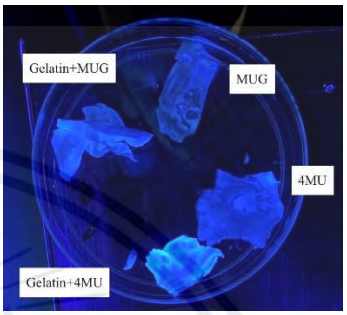
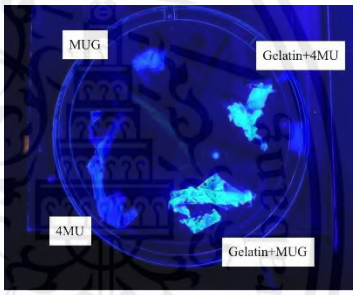
Container	**Product**
Glass plate	
Beaker	
Glass petri dish	
Plastic petri dish	

Aluminum foil	

4.1.2 GUD-MUG reaction of Polylactic acid (PLA) by coating with gelatin 500μM MUG

The product was split into 2 categories which are 4MU and MUG and gelatin mixed with MUG and 4MU. The gelatin is used to enhance the absorbability ability of the film as the gelatin has a great property of absorbing water. Moreover, the film coated with 4MU, gelatin mixed with 4MU were the control group of this experiment. After we observed the result samples under the UV light, the samples coated with 4MU and MUG showed some fluorescence. However, for the samples coated with gelatin mixed with 4MU and MUG showed more fluorescence as shown in table 7. This is because gelatin can absorb more water. The experiment was also conducted on the film produced from glass petri dish and glass plate. Looking into the result of the film produced from these two containers, thinner texture film produced by pouring on glass plate showed more fluorescence product compared to the film produced by pouring on glass petri dish. While observing the fluorescence result, there are some excess solutions on top of the film so I decided to observe the fluorescence result from the excess solution as well. After observing the excess solution under the UV-light, it showed some fluorescence as shown in table 7. This indicates that some of the MUG solution leaked out from the film and GUD-MUG reaction occurred outside the surface of PLA film.

Table 7. GUD-MUG reaction of Polylactic acid (PLA) film

Condition	Product
Product of PLA film produced from glass petri dish	
Product of PLA film produced from glass plate	
Excess solution including MUG leaked out from the film	

4.2 Polylactic acid (PLA) synthesis by microwaving technique

4.2.1 Film fabrication from Polylactic acid (PLA) synthesis by microwaving technique

The film fabrication from Polylactic acid (PLA) synthesis by used microwaving techniques for condense the lactic acid to polymerize the PLA. The sample's consistency became more solidified and viscous than its typical texture after it placed into 90℃ oven for 4 hour and 30

minutes as shown in the figure 29. Even though the sample was prepared at a high temperature, it had to be cooled down to the room temperature before use. However, the PLA synthesis sample after annealed was sticky and could not remove from the paper.

Figure 29. PLA synthesis after annealed was sticky and could not remove from paper

4.2.2 GUD-MUG reaction of Polylactic acid (PLA) synthesis by microwaving technique

The product was separate into 2 categories which were the product without adjusting pH, which was in pH of 3 and the product that adjust the pH to 6. For the first product with pH of 3, no fluorescence was observed under UV-light as shown in the figure 30. This could be due to the lower pH of the sample affects the reaction of GUD. The suitable pH for GUD-MUG reaction is typically between 5.5 to 7.5, and the pH below or above this range reduces the reaction rate and fluorescent intensity [21]. Therefore, the product with pH of 6 indicates more fluorescence product after observed under the UV-light as shown in figure 31. In summary, the film fabrication from PLA synthesis by microwaving technique was not suitable for *E. coli* detection film fabrication according to the result of very low fluorescent product of 4MU.

Figure 30. Fluorescence product from the film with pH 3

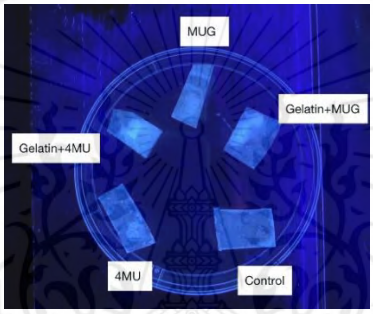


Figure 31. Fluorescence product from the film with pH 6

4.3 Agar-Glycerin

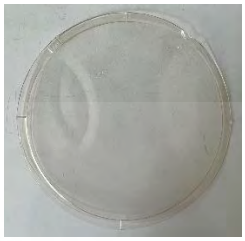
4.3.1 Film fabrication from agar-glycerin

The film fabricated from medium range concentration of agar and used glycerin as a plasticizer of all 3 conditions (Table 5) show a similar texture and thickness as shown in table 8. The film fabricated from medium range concentration visualized in thin transparent film with some strength. To investigate the best condition for *E. coli* detection film fabrication, measuring tensile strength and fluorescent intensity of each condition were required.

Table 5. 20 mL of Medium range solution concentration

Condition	Agar	Glycerin	MUG	Distilled water
1	0.6 g	0.25 mL	250μM, 10 mL	9.75 mL
2	0.4 g	0.376 mL	250μM, 10 mL	9.624 mL
3	0.6 g	0.376 mL	250μM, 10 mL	9.624 mL

Table 8. Film fabrication from 20 mL of medium range solution concentration

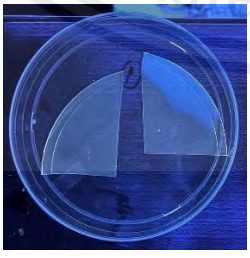
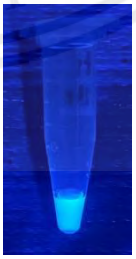
Condition	Product
1	
2	
3	

4.3.2 Fluorescent intensity of Agar-Glycerin film

The resulted product from each condition under the medium range concentration were observed under the UV-light and measured the fluorescent intensity in MATLAB program. The result of all 3 conditions as shown in the table 9, 10 and 11, respectively. The fluorescent intensity were getting on the film that removed excess GUD solution and the MUG solution leaked out from the film. The excess solution on top of the film was also measured as well to ensure whether the MUG solution was leaked out from the film or not. Looking into the graph comparing all 3 conditions as shown in the figure 32, the results of fluorescent intensity product showed that condition 1 has the highest fluorescent intensity comparing to the other 2 conditions. Moreover,

the fluorescence intensity of condition 2 and 3 seem to be fluctuated at reaction time more than 20 minutes. However, the fluorescent product of condition 2 and 3 show the similar intensity as shown from the graph in figure 33.

Table 9. Fluorescence intensity measured from condition 1

Condition 1	**Fluorescence result occur on the film**	**Fluorescence from MUG leaked from the film**	**Intensity**
5 minutes			323007
10 minutes			326481
20 minutes			327665

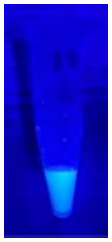
30 minutes			361814

Table 10. Fluorescence intensity measured from condition 2

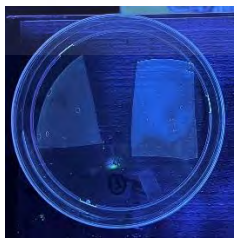
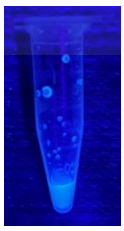
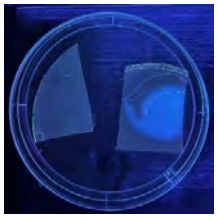
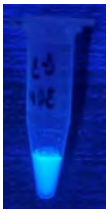
Condition 2	Fluorescence result occur on the film	Fluorescence from MUG leaked from the film	Intensity
5 minutes			314593
10 minutes			320617
20 minutes			318290
30 minutes			309600

Table 11. Fluorescence intensity measured from condition 3

Condition 3	Fluorescence result occur on the film	Fluorescence from MUG leaked from the film	Intensity
5 minutes			307694
10 minutes			314833
20 minutes			319705
30 minutes			318944

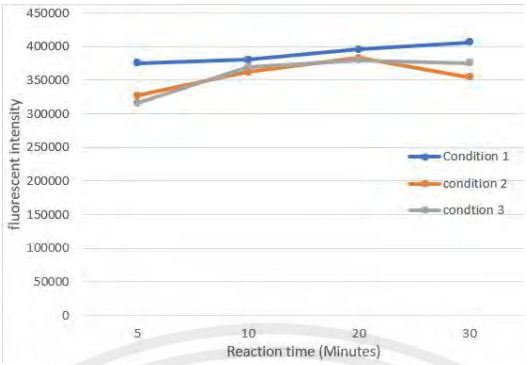


Figure 32. Fluorescent intensity of the film that removed excess solution

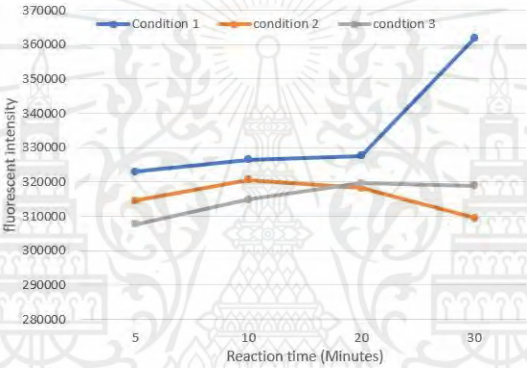


Figure 33. Fluorescent intensity of MUG leaked out from the film

4.3.3 Tensile strength of Agar-Glycerin film

The data collected from the tensile strength machine of all 3 conditions under the medium range concentration were graphically plotted as shown the figure 34. Condition 1 has the highest strength among 3 conditions which the final displacement after pulled by the machine of condition 1 is 13.98 mm. As this condition has the highest amount of agar and lowest concentration of glycerin. For the condition 2 shown in the figure 35, this condition has the lowest strength among 3 conditions which the final displacement after pulled by the machine of condition 2 is 5.16 mm. This is because condition 2 has the lowest concentration of agar. Lastly in the figure 36, condition 3 has the highest elasticity among 3 conditions which the final displacement after pulled by the machine is 15.20 mm. This is because condition 3 contain highest amount of both agar and glycerin.

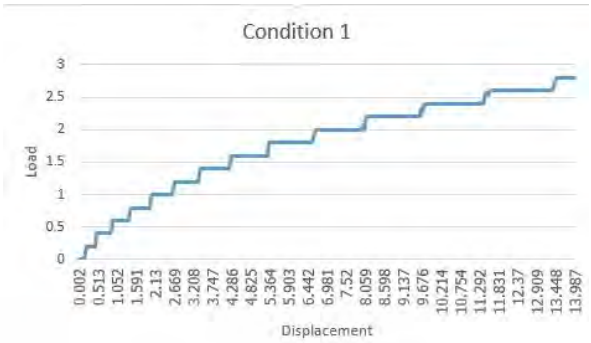


Figure 34. Tensile strength graph of condition 1

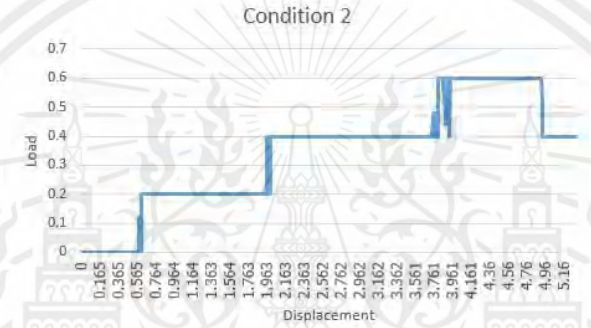


Figure 35. Tensile strength graph of condition 2

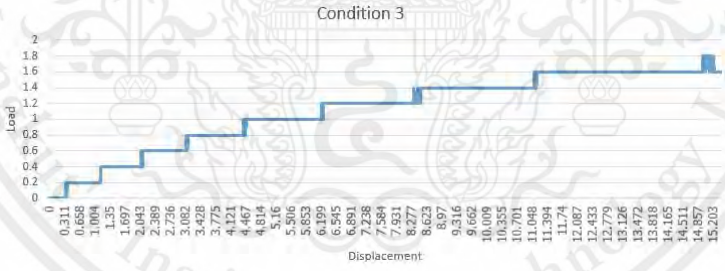


Figure 36. Tensile strength of condition 3

The value of stress, strain and Young's modulus of all 3 conditions were also calculated as well. The tensile strength machine was set to collect 10 data per 1 second which means that there were more than 100 data collected from each condition. The equation below was used to calculate the value of stress, strain and Young's modulus on of EXCEL program. The Young's modulus

values were calculated by using the value of maximum stress divided by maximum strain. The value of Young's modulus of all conditions were 0.0477, 0.0179 and 0.0247, respectively.

Young's modulus calculation by using maximum stress and strain:

$$E = \frac{Max\ stress}{Max\ strain} = \frac{0.0333}{0.6994}$$

$$E = 0.0477 \quad \text{(condition 1)}$$

$$E = \frac{Max\ stress}{Max\ strain} = \frac{0.0047}{0.2653}$$

$$E = 0.0179 \quad \text{(condition 2)}$$

$$E = \frac{Max\ stress}{Max\ strain} = \frac{0.0190}{0.7702}$$

$$E = 0.0247 \quad \text{(condition 3)}$$

Comparing all data from each condition, the condition 1 showed the highest values of Young's modulus and strength. Even though, the final displacement of condition 3 is 15.20 mm which was slightly higher than condition 1 but the value of Young's modulus of condition 1 is higher. Moreover, condition 2 showed the lowest strength according to its final displacement and Young's modulus value which were lower than the other 2 conditions.

CHAPTER 5

CONCLUSION

In chapter 5, the results related to this research was summarized. The suitable polymer and process of film fabrication was calculated, along with the factors which affect the film properties. This conclusion summarized the result related to the objectives of this research, along with the recommendation for the improvement.

5.1 The *E. coli* detection film fabricated

According to the results of PLA dissolved in ethyl acetate and PLA synthesis by microwaving techniques showed that this polymer was not suitable for *E. coli* detection film fabrication. This is because the film fabricated from PLA appeared in a thin white powdery solid which the film should be clear transparent film. For the result of film fabrication of PLA synthesis by microwaving techniques indicated that even though the film was prepared with high temperature, the solution coming out as sticky solution which cannot be removed from the paper. In this experiment, the medium range concentration of Agar-Glycerin at was the optimal biopolymer for film fabrication, as observed from the results in table 8. Furthermore, the experiments detailed in table 9, 10 and 11 provide support for the conclusion that *E. coli* detection film can be fabricated from Agar-Glycerin.

5.2 The optimal ratio for *E. coli* detection film fabrication from Agar-Glycerin

In this experiment, there was 3 conditions in medium range concentration of Agar-Glycerin as shown in table 5. Based on these results, the florescent intensity from each conditions showed that condition 1 has the highest fluorescent intensity comparing to the other 2 conditions. The result of tensile strength of Agar-glycerin film also provide support to this conclusion as well. This is because condition 1 showed the highest tensile strength among 3 conditions which the final displacement after pulled by the machine of condition 1 is 13.98 mm. Even though, the final displacement of condition 3 is 15.20 mm the value of Young's modulus getting from condition 1 which is 0.0477, is highest among 3 conditions.

From the results and experiments of fabricating the *E. coli* detection film by Agar-Glycerin. In accordance with the results of fluorescent intensity and mechanical properties of the film fabricated from Agar-Glycerin. The optimal ratio for *E. coli* detection film fabrication was condition 1 from medium range concentration of Agar-Glycerin at 0.6 g agar and 0.25 mL glycerin in 10 mL total solution.

5.3 Suggestions

For the suggestions to enhance the properties of the *E. coli* film involve the process of film fabrication and GUD-MUG reaction. During the process of film fabrication, drying the solution inside the 40℃ oven is important in order to prevent any contamination to occur on the film. Another suggestion is to investigating more about chemical functional group to trap MUG storing in the film.

REFERENCES

[1] R. L. Buchanan and M. P. Doyle, “Foodborne disease significance of Escherichia coli O157:H7 and other enterohemorrhagic E. coli,” *Food Technol.*, vol. 51, no. 10, pp. 69-76, 1997.

[2] Treebupachatsakul et al., “Gelatin-Based Microfluidic channel for quantitative E. coli detection using blue fluorescence of 4-Methyl-Umbelliferone product and a smartphone camera”, *Sensors*, vol. 22, no. 13, pp. 1-11, July. 2022.

[3] S. Kalyani, B. Bindu and K. Veluraja, “Perspectives of polylactic acid from structure to applications”, *Polymers from Renewable Resources,* vol. 12, pp. 60-74, 2021.

[4] D. da Silva et al., “Biocompatibility, biodegradation and excretion of polylactic acid (PLA) in medical implants and theragnostic systems”, *Chemical engineering journal*, 2018.

[5] L. Ranakoti et al., “Critical review on polylactic acid: Properties, structure, processing, bio composites, and nanocomposites”, *MDPI*, 2022.

[6] T. Casalini et al., “A Perspective on Polylactic Acid-Based Polymers Use for Nanoparticles Synthesis and Applications”, vol. 7, pp. 3-5, October. 2019

[7] L. Gaget, “What is PLA plastic filament and how to use it for 3D printing?,” Sculpteo, [Online]. Accessed Oct. 28, 2023 https://www.sculpteo.com/en/materials/fdm-material/pla-material/#:~:text=This%203D%20printing%20filament%20is,PLA%20is%20exposed%20to%20sunlight

[8] “Agar-agar,” AgarGel, [Online]. Accessed Oct. 28, 2023 https://agargel.com.br/en/agar-agar/#:~:text=In%20its%20gelling%20power%2C%20agar,and%20sharp%20melting%2Fsetting%20temperatures

[9] J. Praiboon et al., “Physical and chemical characterization of Agar Polysaccharides Extracted from the Thai and Japanese Species of Gracilaria”, pp. 11-17, 2006.

[10] "Agar: 9002-18-0," ChemicalBook, [Online]. Accessed Oct. 28, 2023 https://www.chemicalbook.com/ChemicalProductProperty_EN_CB6704452.htm#:~:text=Chemical%20Properties,-Agar%20is%20produced&text=Agar%20contains%20two%20polysaccharides%20%2D%20agarose,are%20methylated%20at%20C%2D6.

[11] M. (Marine Hydrocolloids), "Agar Agar in pharmaceuticals: A revolutionary addition to modern medicine," LinkedIn, [Online]. Accessed Oct. 28, 2023 https://www.linkedin.com/pulse/agar-pharmaceuticals-revolutionary-addition-modern

[12] "Gelatin, characteristics and uses in the food industry," Halal Food Quality, [Online]. Accessed Oct. 28, 2023 https://www.halalfoodquality.com/en/la-gelatina-caracteristicas-y-usos-en-la-industria-alimentaria/

[13] K. Deshmukh et al., "Biopolymer Composites with High Dielectric Performance: Interface Engineering", September. 2016.

[14] F. Milano et al., "Structure and properties of gelatin," Encyclopedia, [Online]. Accessed Oct. 28, 2023 https://encyclopedia.pub/entry/44735

[15] "Gelatin," Uses, Interactions, Mechanism of Action | DrugBank Online, [Online]. Accessed Oct. 28, 2023 https://go.drugbank.com/drugs/DB11242

[16] "What is glycerin: Definition, formation, properties, uses, examples," Toppr, [Online]. Accessed Oct. 28, 2023 https://www.toppr.com/guides/chemistry/chemical-bonding-and-molecular-structure/glycerin/

[17] L. Martins Perin et al., "Glucuronidase activity of escherichia coli isolated from chicken carcasses," Brazilian journal of microbiology: [publication of the Brazilian Society for Microbiology], [Online]. Accessed Oct. 28, 2023 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3768652/#:~:text=The%20%CE%B2%2DD%2Dglucuronidase%20(,EC%20(3M%20Microbiology%2C%20St

[18] G. Malhotra, "Top 4 factors affecting tensile strength test results," prestogroup, [Online]. Accessed Nov. 9, 2023 https://www.prestogroup.com/blog/top-4-factors-affecting-tensile-strength-test-results/

[19] “Young’s modulus and tensile strength: All you need to know,” Sculpteo, [Online]. Accessed Jan. 6,2024 https://shorturl.at/jrsBW

[20] V. Ibarra et. al., “Agar Biopolymer Films for Biodegradable Packaging: A Reference Dataset for Exploring the Limits of Mechanical Performance” *Materials,* pp 4-5, June.2022

[21] M. Groeneveld, N. Catalan, K. Einarsdottir, A. G. Bravo and D. N. Kothawala, “The influence of pH on dissolved organic matter fluorescence in inland waters” *Anal. Methods,* pp. 1351-1360, March. 2022

[22] J. Doh et al. “Back-propagation neural network-based approximate analysis of true stress-strain behaviors of high-strength metallic material” *Journal of mechanical science and technology*, pp. 1234-1235, 2016.

[23] “Polylactic acid MW 60,000 26100-51-6,” Mw 60,000 26100-51-6, [Online]. Accessed Nov. 16, 2023 https://www.sigmaaldrich.com/US/en/product/ALDRICH/

[24] C. Elisa et al., “Poly-L-Lactic Acid (PLLA)-Based Biomaterials for Regenerative Medicine: A Review on Processing and Applications”, *Polymers*, 2022

[25] A. M. Research, “3D printing filament market share, challenges, opportunities and key market trends report by 2023-2032,” openPR.com, [Online]. Accessed Nov. 15, 2023 https://www.openpr.com/news/3260602/3d-printing-filament-market-share-challenges-opportunities

[26] M. L., “BASF & Natural Machine to create customized 3D-printed skincare masks & eye patches,” 3Dnatives, [Online]. Accessed Nov. 15, 2023 https://www.3dnatives.com/en/basf-natural-machine-3d-printed-skincare-masks-eye-patches-181020214/

[27] “100% compostable PLA biodegradable film, polylactic acid film for Packing Bag,” China good quality [#g1#] on sales, [Online]. Accessed Nov. 15, 2023 https://www.shrinkfilmroll.com/sale-9553643-100-compostable-pla-biodegradable-film-polylactic-acid-film-for-packing-bag.html

[28] R. Marwah et al., “Bio-Based Polymer Electrolytes for Electrochemical Devices: Insight into the Ionic Conductivity Performance”, *Materials*, 2020

[29] S. Badgujar, “Global casein peptone market is expected to grow at a CAGR of 4%,” Medium, [Online]. Accessed Nov. 15, 2023 https://medium.com/@sanket482020/global-casein-peptone-market-is-expected-to-grow-at-a-cagr-of-4-2cdd664bdd44

[30] “How to make agar agar cake recipe,” Slurrp, [Online]. Accessed Nov. 15, 2023 https://www.slurrp.com/recipes/agar-agar-cake-1611348460

[31] Bayer, “How Europe can become a leading biotech hotspot,” Bayer Global, [Online]. Accessed Nov. 15, 2023 https://www.bayer.com/en/news-stories/how-europe-can-become-a-leading-biotech-hotspot

[32] “Gelatin,” Amstel Products, [Online]. Accessed Nov. 15, 2023 https://amstelproducts.nl/product/gelatin/

[33] A. Osama, “Drug Delivery of Gelatin Nanoparticles as a Biodegradable Polymer for the Treatment of Infectious Diseases: Perspectives and Challenges”, *Polymers,* 2023

[34] F. Milano et al., “Current trends in gelatin-based drug delivery systems,” MDPI, [Online]. Accessed Nov. 15, 2023 https://www.mdpi.com/1999-4923/15/5/1499

[35] “Clinical guidelines for antidepressant dosage must reflect the evidence,” Department of Psychiatry, [Online]. Accessed Nov. 15, 2023 https://www.psych.ox.ac.uk/news/clinical-guidelines-for-antidepressant-dosage-must-reflect-the-evidence

[36] “Fresh products for your soul,” Kwo Organic Foods, [Online]. Accessed Dec. 1, 2023 https://kwo-organicfoods.com/

[37] “Glycerol (trihydroxypropane): Endogenous metabolite: Medchemexpress,” MedchemExpress.com, [Online]. Accessed Dec. 1, 2023 https://www.medchemexpress.com/Glycerol.html

[38] “4-methylumbelliferyl-β-D-glucuronide hydrate (MUG): Fluorogenic substrate: Medchemexpress,” MedchemExpress.com, [Online]. Accessed Nov. 15, 2023

https://www.medchemexpress.com/4-Methylumbelliferyl-_beta_-D-glucuronide_hydrate.html