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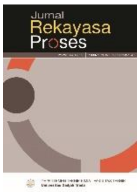

# The Effect of Hydrochloric Acid Solution and Glycerol on The Mechanical, Hydrate Properties and Degradation Rate of Biofilm from Ripe Banana Peels

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## ABSTRACT

Banana peel is a biomass waste that has not been utilised optimally, despite its high starch content. Moreover, starch has potential as a raw material for biofilm or edible film production. This research focused on using the starch content from the mature banana peel to create a biofilm. Starch was extracted from the banana peel; then, it was hydrolyzed with a variation of hydrochloric acid solution (HCl) of 0.5 M (0, 2, 4 %-v/v Starch). Glycerol (0, 20, 40 %-w/w starch) was used as a plasticizer. It was found that the formulation of 4%-v/v HCl solution and glycerol 20%-w/w resulted in the highest biofilm's tensile strength of 4.18 MPa. However, the elongation break percentage achieved the best result at 20,2% when the formulation of 0%-v/v HCl solution and 40%-w/w glycerol was applied. Increasing HCl solution and glycerol was proven to improve the biofilm's solubility in the water, where 47.9% solubility was attained in the formulation of 40%-w/w glycerol and 4%-v/v HCl solution. The degradation rate of biofilm in the soil was measured using zero- and first-order kinetic rates. The zero-order resulted in the best model with a half-life time ($t_{1/2}$) between 73 to 108 days.

Keywords: banana peels; biofilm; degradation rate; film; glycerol; hydrochloric acid; mechanical properties

## *ABSTRAK*

*Kulit pisang merupakan limbah biomassa yang belum dimanfaatkan secara optimal, padahal kulit pisang memiliki kandungan pati yang tinggi. Pati juga berpotensi digunakan sebagai bahan baku dalam pembuatan bioplastik atau edible film. Pada penelitian ini, pati dari kulit pisang matang digunakan sebagai bahan baku pembuatan bioplastik. Pati diesktrak dari kulit pisang, lalu dihidrolisis dengan larutan asam klorida (HCl) 0,5M (0, 2, 4%-v/v pati). Gliserol (0,20, 40 %-b/b pati) digunakan sebagai plasticizer. Hasil penelitian ini menunjukkan bahwa formulasi larutan HCl 4%-v/v dan gliserol 20%-b/b memberikan bioplastik dengan tensile*

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*strength tertinggi, yaitu sebesar 4,18 MPa. Persentase elongasi terbaik didapatkan sebesar 20,2% ketika formulasi larutan HCl 0%-v/v dan gliserol 40%-b/b. Meningkatkan larutan HCl dan gliserol terbukti dapat meningkatkan kelarutan bioplastik di dalam air. Sekitar 47,9% kelarutan didapatkan pada formulasi gliserol 40%-b/b dan larutan HCl 4%-v/v. Laju degradasi bioplastik dihitung dengan kinetika orde nol dan orde satu. Kinetika orde nol memberikan model terbaik dengan waktu paruh ($t_{1/2}$) sekitar 73–108 hari.*

*Kata kunci: asam klorida; bioplastik; film; gliserol; kulit pisang; laju degradasi; properti mekanik*

## 1. Introduction

Indonesia is one of the largest agricultural countries. It is predicted that Indonesia produces 200 million tons of agricultural waste annually (Pranoto et al., 2013). Unfortunately, these agricultural wastes have not been utilised optimally. Banana is one of the agricultural products that has continuous growth every year. In 2020 around 7.425 million tonnes of bananas were produced, while 82 thousand tons were left as waste (Susilawati and Wahyuningsih, 2020). Banana peel comprises around 35%-w of banana, and it contains 20–39% starch, depending on the banana variety, maturity, and growth environment (Giri et al., 2016; Ravi and Mustaffa, 2013). Unfortunately, despite containing starch, banana peel utilisation is still limited.

Starch is a natural polysaccharide found in plants that have been used for various functions (such as thickener and binders) due to its properties. Starch comprises two main polymers, amylose, and amylopectin. Amylose is a linear polymer $\alpha-1{,}4$ anhydroglucose units; while amylopectin is a branched polymer of $\alpha-1{,}4$ chains linked by $\alpha-1{,}6$ glucosidic branch in every 25–30 glucose units (Jenkins and Donald, 1998; Jiménez et al., 2012). Starch can be used as a base matrix to create film or biofilm. However, starch usually has higher amylopectin content than amylose content. Amylose and the branch of amylopectin create an amorphous region, while amylopectin forms a rigid crystalline region. According to Romero-Bastida et al. (2005), amylose influences the ability to produce a film. Thus, starch modification is likely to be implemented to increase the ratio of amylose to amylopectin. Acid modification using hydrochloric acid or acetic acid solution is often used to induce the transformation of amylopectin to amylose (Zhang et al., 2019). This process eventually alters the psychochemical properties of starch that benefit biofilm making without changing the starch granule structure (Pratiwi et al., 2018). Thus, biofilm is often mixed with plasticizers to have suitable mechanical, hydrate, and biodegradable properties. Glycerol is one most used plasticizers to increase the flexibility of biofilm. It has low molecular weight and high hydrophilicity. Adding glycerol to the main biopolymer will modify the starch matrix and enhance the mobility of the polymer chain (Krochta, 2002). These psychochemical properties make biofilm suitable as food packaging to replace conventional plastic (Guilbert et al., 1997; Han, 2014).

Various works related to the generation of starch-based biofilm or edible films have been published. Zhang et al. (2019) reported that acid hydrolysis allows the specific film properties to be elicited on the psychochemical properties of pea starch film. Increasing hydrolysis time resulted in the decrease of the molecular weight of amylopectin and the increase of biofilm's tensile strength. Maniglia et al. (2019) studied different methods of starch modification (water, acid, and alkali) and the type of plasticizers for babassu starch-based film. It was found that acid and alkali modifications plasticized with sorbitol generated high mechanical resistance. Illing et al. (2019) and Robiah (2020) utilised banana peel starch with the addition of additives (ZnO, cellulose, chitosan, and clay). Supplementing the additives into formulation has proven to improve the tensile strength of the film. Bebartta et al. (2020) employed glycerol as a plasticizer (5-20%w/w). Increasing glycerol thickened the banana peel starch based-film and enhanced the film's peel ability and flexibility. Purbasari et al. (2020) varied the type and concentration of plasticizer (30-70%w/w) in the production of banana peel starch based-film. Glycerol was proven to generate film with better mechanical resistance compared to sorbitol. Tarique et al. (2021) reported the arrowroot-starch based film using solution casting technique by varying glycerol (15–45%w/w). It was found that glycerol increased thermal stability and decreased the brittleness and fragility of the film. Taweechat et al. (2021) produced edible packaging film from banana and banana peel starch to extend the shelf life of minced pork. The edible film was prepared using glycerol 25%w/v. The obtained edible film managed to maintain lipid oxidation but not necessarily inhibit microbial growth. Medeiros Silva et al. (2020) also prepared edible film from a mix of banana peel, corn, and loquat leaf starch with 14%w/w glycerol. They indicated 98–100% biodegradability of edible film. This biodegradability was measured for 40 x 40 $mm^2$ film after ten days of soil burial.

Despite various research, unfortunately, there is still little information regarding the influence of acid modification on the banana peel starch-based film properties and the kinetic rate of biofilm degradations. Thus, this research covers those topics in the study. The research was focused on the production of biofilm from overripe banana peel. The ripe banana peel was selected since most of the agricultural wastes were often found overripe. Therefore, the starch' amount and quality in ripe banana peels were analysed to understand its effect on biofilm properties. The amount of hydrochloric acid solution (0, 2, 4%-v/v) and glycerol (0, 20, 40%-w/w) were varied to determine biofilm properties. The mechanic and hydrate properties were measured to understand the quality of biofilm. The biodegradation rate was calculated based on zero- and first-order kinetic.

## 2. Research Methodology

### 2.1 Materials

Ripe *saba banana / pisang kepok* (*Musa acuminata*) peels were used in this research. Saba banana was attained from a local market in Ciumbuleuit, Bandung. Sodium meta bisulfate ($Na_2S_2O_5$; 99% purity Merck, Germany) extracted starch from banana peel. Solution of hydrochloric acid (HCl; 37% purity Merck, Germany), potassium hydroxide (NaOH; anhydrous 98% purity Sigma-Aldrich, USA), and anhydrous glycerol (99.9% purity Sigma-Aldrich, USA) were used for creating a biofilm.

Materials used for starch content analysis were HCl 3% (Merck, Germany), NaOH 30% (Brataco Chemika, Indonesia), $CH_3COOH$ 3% (Brataco Chemika, Indonesia), KI 20% (Brataco Chemika, Indonesia), $H_2SO_4$ 25% (Merck, Germany), and $Na_2S_2O_3$ 0.1 N (Merck, Germany). As for amylose analysis, materials used were ethanol 95% (Merck, Germany), NaOH 1 N (Brataco Chemika, Indonesia), $I_2$ 2% (Brataco Chemika, Indonesia), and $CH_3COOH$ 1 N (Brataco Chemika, Indonesia). The soil for degradation test was bought online (Media Tanam Organik). It contained fertile soils from Lembang, Bandung.

## 2.2 Procedures

### 2.2.1 Starch Extraction from Banana Peels

The method of the starch extraction process was modified from Hadisoewignyo et al. (2017). Banana peels (350 g) were washed with running water to remove impurities and cut into smaller sizes. The solution of sodium meta bisulfate 0.5%-w (240 mL) and freshwater (750 mL) were added into the banana peels, then mixed in the food processor (Phillips; speed 2; 5 minutes). The blended banana peel was filtrated using a cheesecloth, and the filtrate was kept for 72 hours to obtain starch sediment. Starch sediment was filtered and subsequently dried in the oven for 60 °C and 24 hours.

### 2.2.2 Starch Composition

The water content in the dry starch sediment was measured using a moisture analyser (Mettler Toledo). The starch content was determined following the standardized method (SNI 3451:2011) by BSNI (2011). Around one gram of sample was put into a 100 mL Erlenmeyer. Solution of HCl 3%-w (40 mL) was added and refluxed for 3 hours using a condenser. The solution was cooled and neutralized with 30%-w NaOH solution, then a few drops of $CH_3COOH$ 3%-w solution were added. The solution was moved into a 100 mL volumetric flask then distilled water was added until it reached the volume mark. Afterwards, the solution was filtered, and 10 mL of sample was put into Erlenmeyer. Around 25 mL of Luff solution and 15 mL of distilled water were added, then the solution was heated until the boiling point for 10 minutes and then quenched in the ice. After the solution was cooled, 15 mL of 20%-w KI solution and 25 mL of 25%-w $H_2SO_4$ solution were added slowly. The solution was titrated using $Na_2S_2O_3$ 0.1 N solution until the solution turned from purple/blue to pale yellow. The amount of $Na_2S_2O_3$ 0.1 N solution used was measured, then the weight of glucose was subsequently determined using Equation (1). The starch content and yield were calculated with Equation (2) and (3).

$$\text{Glucose content} = \left(\frac{W_s \times fp}{W_g}\right) \qquad (1)$$

$$\text{Starch content} = 0{,}9 \text{ x Glucose} \qquad (2)$$

$$\text{Yield (\%)} = \frac{mass\ of\ starch\ extracted\ (g)}{mass\ of\ banana\ peels\ (g)} \times 100\% \qquad (3)$$

$W_s$ is the weighted sample (mg). $W_g$ is the weight of glucose (mg) based on the data provided by BSNI (2011), where the glucose obtained was 22.85 mg. *Fp* is the dilution factor of 0.01.

The amylopectin was measured by the iodine colorimetric method (Juliano, 1971). Around 0.1 g sample was added into a 100 mL flask and mixed with 1 ml ethanol 95%-w solution and 9 mL NaOH 1N solution. The solution was heated in a 100 °C water bath for 10 minutes then cooled for 1 hour. After cooled, the solution was diluted with distilled water until the volume was 100 mL. Around 5 mL of the diluted solution was added into a 100 mL flask. The solution of 1N acetic acids (1 mL) and 2 mL of $I_2$ 2%-w solution was added into the sample; the solution was diluted with distilled water up to 100 mL. The solution was shaken until homogenous and kept for 20 minutes. The absorbance of the blue sample was measured at 630 nm wavelength using UV-Vis Spectrophotometer (Thermo Scientific Genesys 20). The concentration of amylose was measured using Equation (4). The amylose content was calculated using Equation (5). The amylopectin content was measured by Equation (6).

$$\text{Absorbance (A)} = 0.8613\ \text{C (mg/mL)} \tag{4}$$

$$\text{Amylose content (\%)} = C \times V \times fp \times 100\% \tag{5}$$

$$\text{Amylopectin content (\%)} = \text{Starch content (\%)} - \text{Amylose content (\%)} \tag{6}$$

*C* is the amylose concentration (mg/mL), *V* is the sample volume (mL), and *fp* is the dilution factor of 0.02.

### 2.2.3 Production of Biofilm

The procedure for biofilm production was formulated based on Gaonkar et al. (2018) and Jayachandra et al. (2016). The dried starch sediment was stirred into distilled water, with the ratio of starch to water being 0.04 w/w. The solution was mixed and heated at 80 °C for 5 minutes at 600 rounds per minute (rpm). Solution of hydrochloric acid (0, 2, 4%-v/v) was added into the solution at 80 °C for 15 minutes at 600 rpm. The solution was then neutralized using 0.5M NaOH solution. Glycerol (0, 20, 40%-w/w) was added as a plasticizer. The mixture was then blended and heated at 80 °C for 15 minutes at 600 rpm. Biofilm solution was poured into a Petri dish, and then it was dried in the oven at 60 °C for two hours.

### 2.2.4 Biofilm Characterisation

The thickness of biofilm was measured by a digital calliper (Mitutoyo, Japan). The mechanical properties (tensile strength and elongation break) were measured using Texture Analyzer (CT3 Brookfield, USA). Biofilm was sliced into a size of 6 x 2 cm. The trigger, initial distance between the grips, and the initial velocity of the Texture Analyzer were adjusted to 7.5 g, 30 mm, and 0.5 mm/s. Mechanical properties were calculated using the average thickness of each biofilm and replicated twice.

The hydration property measured was biofilm' water content and solubility. The method to measure water or moisture content followed the procedure by Basiak et al. (2018). The biofilm was dried at 105 °C for 24 h. The moisture content was calculated with Equation (7). The method to measure solubility property was based on Colla et al. (2006) and Chiumarelli & Hubinger (2014). The biofilm specimen was dried at 110 °C for 24 hours to obtain the initial dry mass. Then, each sample was immersed in the 50 mL distilled water at room temperature for 24 hours and periodically agitated. The sample was dried at 110 °C for 24 hours to obtain the final dry mass. The biofilm water solubility was calculated with Eq. 8, and all samples were performed in duplicate. The ANOVA Two Way testing analysis was also conducted to determine the influence of each variable statistically on mechanical and hydration properties using Microsoft Excel$^{\text{TM}}$ as a tool.

$$\text{Moisture content (\%)} = \left(\frac{W_i - W_f}{W_i}\right) \times 100\% \tag{7}$$

$$\text{Solubility (\%)} = \left(\frac{W_{di}-W_{df}}{W_{di}}\right) \times 100\% \qquad (8)$$

$W_i$ is the initial dry mass (mg) before drying, $W_f$ is the final dry mass (mg) after drying, $W_{di}$ is the initial dry mass (mg) before water immersion, and $W_{df}$ is the final dry mass (mg) after water immersion.

Since the biofilm is used as packaging, biofilm with the highest tensile strength and elongation break will be tested for Fourier Transform Infrared (FTIR; Shimadzu, Japan) and soil degradation rate test. The degradation rate of biofilm was based on a method by Wahyuningtyas and Suryanto (2017) with a modification. The biofilm was cut into 3 x 3 cm. The soil was put into the plastic pot (500 mL), and the sample was buried 2 cm from the surface. The sample's weight was measured every two days for two weeks. The degradation rate of biofilm in the soil was evaluated using zero-order (Equation (8)) and first-order kinetic models (Equation (9)). The most satisfactory model was selected by comparing the coefficient of determination ($R^2$).

$$W = W_o - k_o.t \qquad (8)$$

$$W = W_o \,.\, \exp(-k_1.t) \qquad (9)$$

$W$ is the mass of biofilm (g) at time t, $Wo$ is the mass of biofilm (g) at $t$ = 0, $k_o$ and $k_1$ are the degradation rate constant for zero-order (g biofilm/day) and first-order ($day^{-1}$) model.

## 3. Results and Discussion

### 3.1. Starch Composition

After the extraction process, the obtained starch sediment was dried to reduce the moisture content and resulted in the starch sediment of around 1.15 g/100 g banana peels. The dried starch sediment was used as the primary source for biofilm preparation and required starch and moisture composition testing. The results displayed in Table 1 showed that the sediment contained 82.29% starch and 5.36% moisture. Thus, the starch yield from the overripe banana peel was around 0.95% or 0.95 g per 100 g banana peels. This value is considered very low compared to Li et al. (2018), in which approximately 22% starch was detected on banana peels. However, this result is acceptable since starch content depends on the ripeness of the banana peel. The more mature the banana peel, the starch content can significantly drop to 1% (Zhang et al., 2005). Since the overripe banana peel was used in this research, the low starch yield is still tolerable.

The amylose content was around 22.5% of starch or 0.22% of banana peels. According to Ravi and Mustaffa (2013), the amylose content of various banana peels is around 20–39% of starch total content. Peroni-Okita et al. (2010) reported that the amylose content of Nanicao banana was about 15% of starch total content. The amylose content of the banana peels is influenced by the banana' ripeness, variety, and growth environment. The lower amylose content might be caused by amylose degradation into glucose and sucrose during the maturity of the banana peel (Chang, 1979).

**Table 1.** The dried starch sediment composition

| Content | Value (%-w/w) |
|---|---|
| Moisture | 5.36 |
| Starch | 82.29 |
| - Amylose | 18.58 |
| - Amylopectin | 63.71 |

## 3.2. Biofilm Characterization

### 3.2.1 Mechanical Properties

Biofilm thickness and density obtained from each sample can be seen in Table 2. The thickness of biofilm was the average thickness measurement in three different places. From Table 2, it is discovered that the thickness of biofilm ranges between 0.070–0.163 mm. According to Fakhouri et al. (2013), the average biofilm thickness from corn starch is around 0.15 mm, while Taweechat et al. (2021) produced biofilm from a mixture of banana and banana peel starch with thickness around 0.030–0.047 mm. The thickness of biofilm depends on the particle size of starch granules and the biofilm formulation, which is connected to the free volume of starch or polymer structures. Increasing the acid solution transforms amylopectin into smaller starch to fill the free volume between longer polymer chains and create a denser film. At the same time, glycerol also can relocate itself between the polymer molecules and enhance the free volume, thus increasing the thickness (Zhang et al., 2019).

The biofilm's tensile strength and elongation break profile can be observed in Figure 1 and 2, respectively. The highest value of biofilm's tensile strength was obtained by formulating 4%-v/v of HCl solution and 20%-w/w of glycerol (S8). Increasing the glycerol as a plasticizer ameliorated the tensile strength of biofilm. However, increasing glycerol to 40%-w/w did not necessarily improve the tensile strength, suggesting biofilms were more flexible due to the plasticising effect. The biofilm without glycerol addition tended to be brittle and resulted in low tensile strength. The retrogradation phenomenon occurred during the cooling after the starch was heated and gelatinized. The disaggregated amylose and amylopectin were reconnected to form a more ordered structure. One of the characteristics of starch retrogradation is the increase of degree crystallinity. For long-chain polymer, retrogradation might cause the transformation into a firm gel. However, starch retrogradation is not favoured for short polymer chains due to biofilm's brittleness (Wang et al., 2015). Glycerol is a low molecular weight agent mixed into the polymer chain to improve the thermoplasticity of the polymers. Glycerol can rearrange itself between long polymer chains; thus, adding glycerol into biofilm increases the polymer structure's free volume and inhibits long polymer chains' interaction. Consequently, it overcomes the biofilm's brittleness and increases the flexibility of the polymer (Han, 2014).

**Table 2.** Thickness and density of biofilm

| Sample No. | HCl (%-v/v) | Glycerol (%-w/w) | Thickness (mm) | Density (g/$cm^3$) |
|---|---|---|---|---|
| S1 | 0 | 0 | 0.070 ± 0.051 | 0.292 ± 0.007 |
| S2 | | 20 | 0.115 ± 0.047 | 0.252 ± 0.004 |
| S3 | | 40 | 0.163 ± 0.019 | 0.159 ± 0.0001 |
| S4 | 2 | 0 | 0.143 ± 0.014 | 0.184 ± 0.015 |
| S5 | | 20 | 0.118 ± 0.141 | 0.212 ± 0.013 |
| S6 | | 40 | 0.110 ± 0.094 | 0.231 ± 0.010 |
| S7 | 4 | 0 | 0.133 ± 0.052 | 0.168 ± 0.004 |
| S8 | | 20 | 0.125 ± 0.047 | 0.205 ± 0.004 |
| S9 | | 40 | 0.143 ± 0.033 | 0.183 ± 0.0002 |

The addition of hydrochloric acid solution was to modify starch through acid hydrolysis. The hydroxonium ion from hydrochloric acid attacks the oxygen in the glycosidic bond and induces the hydrolysis process in the linkage. The acid modification increases short linear chains like amylose to retrograde (Pratiwi et al., 2018). Based on Figure 1, the increment of the acid solution improves the tensile strength of biofilm. According to Zhang et al.

(2019) and Cheng et al. (2007), the acid solution could transform the amylopectin into amylose and smaller low molecular weight of starch. The smaller starches could fill the free volume between long polymer chains and effectively cause the biofilm to be denser. This occurrence might lead to the enhancement of the polymer matrix and the increment of biofilm's tensile strength.

An opposite trend of tensile strength profile can be observed in the elongation break values (Figure 2), where naturally, biofilms with high tensile strength have low elongation break values. It can be noticed that the flexibility of biofilm increased as a function of plasticizer content. Due glycerol relocation between the starch structures, the polymer matrix tends to be less dense, and it facilitates the movement of polymer chains (Chiumarelli and Hubinger, 2014).

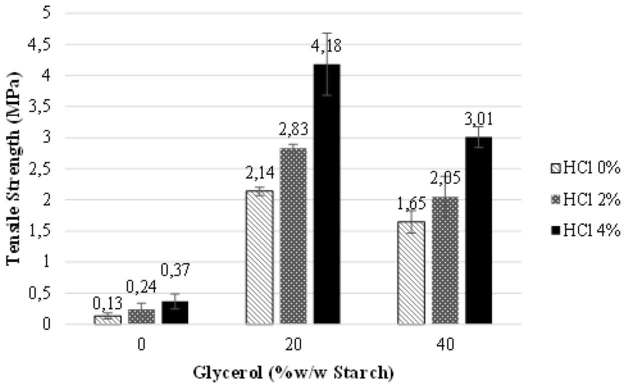


**Figure 1.** The profile of biofilm's tensile strength on various HCl concentration and glycerol

However, the acquired biofilms have not yet accomplished the requirements. According to Han (2014), an excellent commercial edible film should have tensile strength and elongation break equal to or larger than 10 MPa and 10%, respectively. These results might be due to lower starch content in overripe banana peels, and it affected the polymer matrix properties of the biofilm.

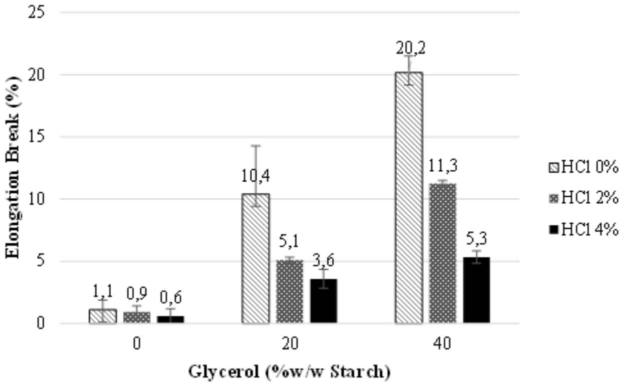


**Figure 2.** The profile of biofilm's elongation percentage on various HCl concentration and glycerol

### 3.2.2 Hydration Properties

The moisture content and solubility of biofilm can be observed in Figure 3 and 4, respectively. It is displayed that increasing glycerol content yielded biofilm with higher moisture or water content. This result follows the outcomes by Han (2014), Cerqueira et al. (2012), and Tarique et al. (2021) that glycerol contains hydroxyl (-OH) groups that attract water molecules. This characteristic of glycerol enables biofilm to confine water and form hydrogen bonds within the starch matrix.

The solubility of biofilm showed the ability of biofilm to be dissolved in the water. From the solubility profile in Figure 4, it can be seen that the solubility of biofilm from banana peel starch ranges from 11.3% to 47.9%. Compared the results to Farahnaky et al. (2013), Chiumarelli and Hubinger (2014), and Utami Hatmi et al. (2020), the results obtained were still within the range. Increasing the glycerol content enhanced the solubility of biofilm. Glycerol is very hydrophilic and hygroscopic; thus, incorporated glycerol into biofilm will form a hydrodynamic plasticizer-water complex and attracts water molecules (Han, 2014). Increasing the acid solution also enhances the solubility of biofilm in the water. It is suggested by Cheng et al. (2007) that the hydrodynamic characteristic of biofilm might be influenced by the number of polar (-OH) groups in the starch matrix. More acid solutions used for hydrolysis would promote the production of more polar chain ends. Hence, it would facilitate the solubility of biofilm. Additionally, the acid solution would transform long starch chains into shorter chains. This transformation might result in the biofilm being easier to dissolve in the water.

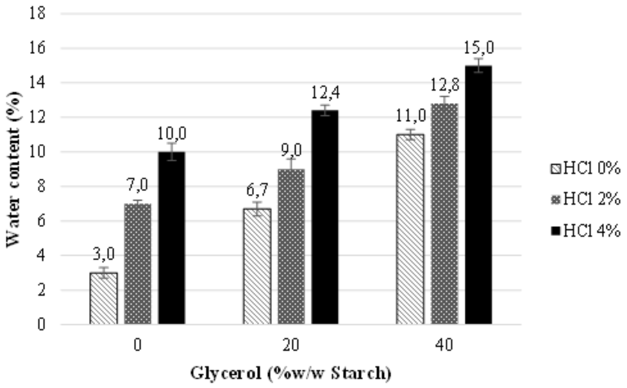


**Figure 3.** The profile of biofilm's water content depends on the HCl concentration and glycerol

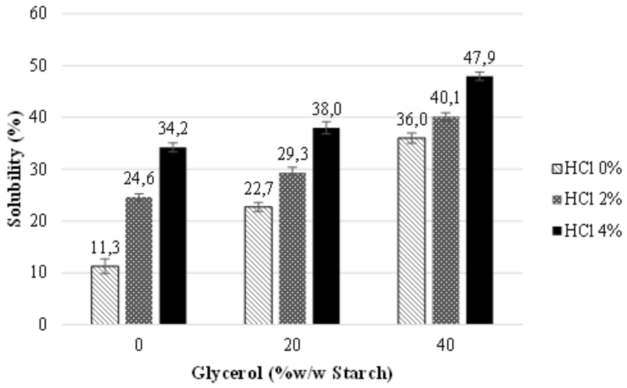
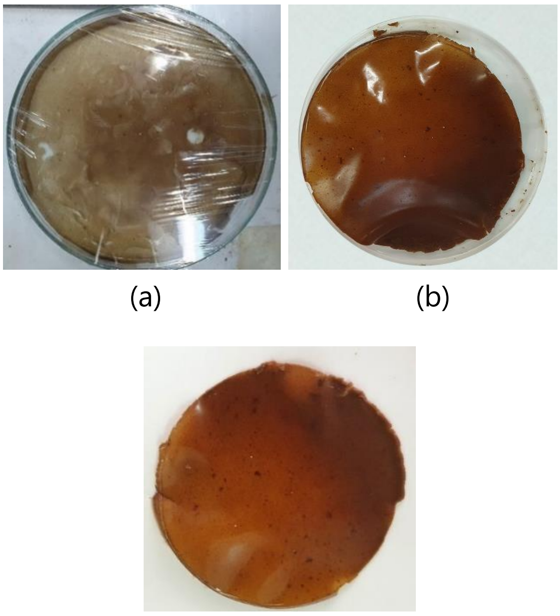


**Figure 4.** The profile of biofilm's solubility in the water depends on the HCl concentration and glycerol



(a) (b)

(c)

**Figure 5.** Biofilm of (a) S1 (HCl 0%v/v & glycerol 0%-w/w), (b) S3 (HCl 0%-v/v & glycerol 40 %-w/w), and (c) S8 (HCl 4%-v/v & glycerol 20%-w/w)

The visuals of biofilm for S1, S3 (best elongation break), and S8 (best tensile strength) were displayed in Figure 5. It can be noticed that without the addition of hydrochloric acid solution and glycerol, biofilm had a thin film and tended to be fragile and brittle. S3 has the thickest film and high stickiness to the container. The stickiness of S3 might be due to high glycerol content (40%-w/w). S8 has lower stickiness than S3, and this property makes peeling the biofilm from the container easier.

### 3.2.3 Statistical Analysis

The ANOVA test was also calculated to determine the influence of variables and their interactions to biofilm properties. The significance of each variable was analysed by comparing the computed F value (F) to the critical F value ($F_{crit}$). When F is larger than the $F_{crit}$, it exhibits the variable's significance. It can be observed from Table 3 that hydrochloric acid (HCl) solution, glycerol content, and both interactions of HCl solution and glycerol indeed have a strong influence on the tensile strength, the elongation break, and the solubility of biofilm.

### 3.2.4 Fourier Transform Infrared (FTIR)

The best samples with the highest tensile strength (S8: HCl of 4%-v/v and glycerol 20%-w/w) and elongation break (S3: HCl of 0%-v/v and 40%-w/w) were selected for FTIR analysis. From Figure 6, the biofilm profile for

**Table 3.** Two way ANOVA statistical analysis performed (α = 0.05)

| Source | Sum of Square | DoF | Mean Square | F | $F_{crit}$ |
|---|---|---|---|---|---|
| **Tensile Strength (TS)** | | | | | |
| HCl Conc. (A) | 4.655 | 2 | 2.327 | 22.30 | 4.256 |
| Glycerol (B) | 19.933 | 2 | 9.966 | 95.48 | 4.256 |
| Interaction (AB) | 1.689 | 4 | 0.422 | 4.04 | 3.633 |
| Error | 0.939 | 9 | 0.104 | | |
| Total | 27.218 | 17 | | | |
| **Elongation (EB)** | | | | | |
| HCl Conc. (A) | 122.583 | 2 | 61.291 | 77.57 | 4.256 |
| Glycerol (B) | 394.787 | 2 | 197.393 | 249.82 | 4.256 |
| Interaction (AB) | 102.574 | 4 | 25.643 | 32.45 | 3.633 |
| Error | 7.111 | 9 | 0.7901 | | |
| Total | 627.055 | 17 | | | |
| **Solubility** | | | | | |
| HCl Conc. (A) | 856.295 | 2 | 428.147 | 954.25 | 4.256 |
| Glycerol (B) | 1017.745 | 2 | 508.872 | 1134.17 | 4.256 |
| Interaction (AB) | 75.167 | 4 | 18.791 | 41.88 | 3.633 |
| Error | 4.038 | 9 | 0.448 | | |
| Total | 1953.247 | 17 | | | |

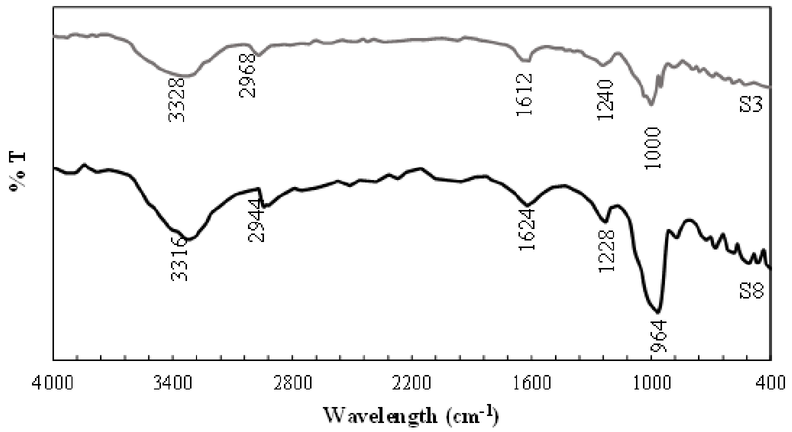


**Figure 6.** FTIR profile of biofilm from banana peels with HCl 4%-v/v and glycerol 20 %w/w (S8) and HCl 0%-v/v and glycerol 40 %w/w (S3)

each sample showed a similar tendency between S3 and S8. According to Basiak et al. (2018), the determining properties of biofilm Is the hydrogen bond due to the transformation of polymer matrix through acid hydrolysis and glycerol addition. It is noted from Figure 6 that both samples have peaks at 33216 (S8) and 3328 $cm^{-1}$ (S3), which showed a stretch of "polymeric" hydroxyl group, which corresponded to the vibration model of OH-groups from the glycerol and absorbed water. However, the peak for S8 is steeper than S3, and it displayed the significance of acid hydrolysis to the formation of the hydrogen bond. The peak at 2944 (S8) and 2968 $cm^{-1}$ (S3) were related to the vibration of the hydrogen bond, but the band values are relatively weak. The peak at 1624 (S8) and 1612 $cm^{-1}$ (S3) showed a stretch of methyl (C-H) and had a weak absorption band. The peak at 964 (S8) and 1000 $cm^{-1}$ (S3) proved the existence of C-OR stretching from the starch.

### 3.2.5 The Degradation Rate of Biofilm in the Soil

The measurement of biofilm's degradation in the soil was applied to the sample with the highest tensile strength (S8: 4%-v/v HCl and 20%-w/w glycerol) and the highest elongation break (S3: 0%v/v HCl and 40%-w/w glycerol). The results can be seen in Figure 7 (a). After sixteen days, 7.2% and 11.5% of S8 and S3 biofilms were disintegrated. Biofilm S3 was faster to degrade compared with biofilm S8 due to higher glycerol concentration. According to Dean et al. (2013), the addition of glycerol as a plasticizer influenced the ability of biofilm to be decomposed by soil microorganisms. Glycerol is very hygroscopic and incorporated more glycerol into biofilm modifies the material permeation and water absorption. Consequently, it affects to faster biodegradation rate.

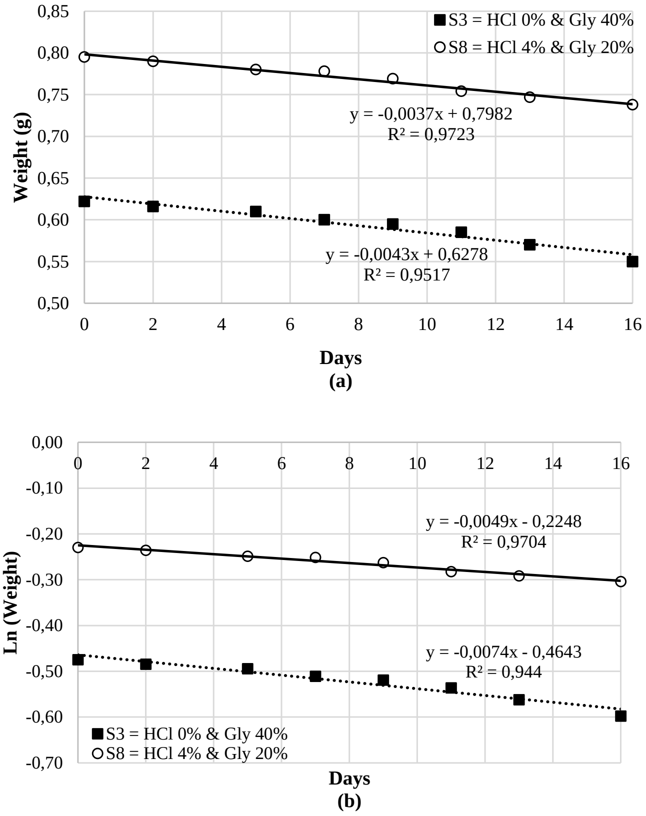


**Figure 8.** Degradation rate profile of biofilm from banana peels using (a) zero-order kinetic and (b) first-order kinetic

**Table 4.** The kinetic degradation rate of biofilm according to zero- and first order ($k_o$ = g biofilm/days & $k_1$ = $days^{-1}$)

| Sample No. | Zero Order | | | First Order | | |
|---|---|---|---|---|---|---|
| | $k_o$ | $R^2$ | $t_{1/2}$ (days) | $k_1$ | $R^2$ | $t_{1/2}$ (days) |
| S3 | 0.0043 | 0.9517 | 73 | 0.0074 | 0.944 | 94 |
| S8 | 0.0037 | 0.9723 | 108 | 0.0049 | 0.9704 | 141 |

In this research, the degradation rate of biofilm was calculated in zero- and first-order kinetic. This analysis was conducted to understand which kinetic rate that representable for biodegradability. From Figure 7 and Table 4, the zero- and first-order kinetic models had no significant difference. However, comparing the coefficient of determination ($R^2$), the zero-order kinetic gave the best model for S8 and S3 with $R^2$ = 0.9723 and 0.9517, respectively. Based on the zero-order kinetics, the half-life times for S3 biofilm was 73 days, while S8 biofilm was 108 days.

## 4. Conclusions

The banana peel is one of the agricultural wastes that has not been used optimally. The starch content in the mature banana peel was low, with an amylopectin to amylose ratio of around 3.5. The starch from banana peel could be utilized to generate biofilm. Due to high amylopectin content, acid modification using hydrochloric acid solution was used to transform amylopectin into amylose or smaller starch molecules. Thus, it creates biofilm with a denser matrix and enhances tensile strength. Glycerol was used as a low molecular plasticizer to enhance the polymer matrix's free volume and facilitate the movement of polymer chains. Therefore, increase the elongation break value of the biofilm. However, the biofilm tensile strength and elongation break were still below the standard of commercial edible film. These results might occur due to the low starch content of the banana peels. Thus, it influenced the weaker polymer matrix. The addition of glycerol increases the solubility of the biofilm and creates a faster degradation rate. The degradation rate of biofilm in the soil follows the zero-order kinetic rate. For future research, the mixture of different plasticizers should be analyzed to lower the solubility of biofilm but still can enhance the mechanical properties of the biofilm. Additionally, the safety of biofilm as food packaging must be ensured. Thus, biofilm's chemical and elemental trace testing are necessary to observe harmful components.

## Notation

$A$ = absorbance of the sample during amylose test, -
$C$ = the amylose concentration, mg/mL
$fp$ = dilution factor, -
$k_o$ = Degradation rate constant for zero-order, g biofilm/day
$k_1$ = Degradation rate constant for first order, day$^{-1}$
$R^2$ = The coefficient of determination, -
$t_{1/2}$ = Half lifetime, days
$T$ = Transmission, %
$V$ = sample volume, mL
$W_s$ = mass of sample during glucose test, mg
$W_g$ = mass of glucose, mg
$W_i$ = initial dry mass before drying, mg
$W_f$ = final dry mass after drying, mg
$W_{if}$ = Initial dry mass of the sample before water immersion, mg
$W_{df}$ = Final dry mass of the sample after water immersion, mg
$W$ = mass of biofilm at time t during degradation test, g
$W_o$ = Mass of biofilm at time t=0 during degradation test, g

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