Comparing the Performance of *Lactobacillus* *delbrueckii* and *Lactobacillus* *rhamnosus* on The Formation of Lactic Acid from Glucose

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Abstract. For producing lactic acid, it may use pure glucose. Lactic acid is a category in organic compounds, and it has a high sales value since being used in the food processing industry is quite diverse. Lactic acid has also has used in numerous manufacturing fields and that same food industry, including pharmaceuticals, cosmetics, chemicals, and textiles. We can make lactic acid from refined starch and cellulose fermentation materials; lactic acid is a monomer of poly-lactic acid (PLA), the primary ingredient manufacturer of environmentally friendly biodegradable plastic. Variables used in this research include up to 10-60 percent for medium BHM (Bushnell Haas Medium), 40-60 percent for medium MRS (de Man Rogosa and Sharpe), and *Lactobacillus delbrueckii* and *Lactobacillus rhamnosus* for glucose concentration. After the fermentation process, we took lactic acid, and absorbance, lactic acid, and glucose levels have measured. The study shows that best-operating conditions are 21 hours of fermentation time. *Lactobacillus delbrueckii* develops more vital lactic acid from both the media used, BHM, and MRS media.

Keywords: glucose, lactic acid, *Lactobacillus delbrueckii*, *Lactobacillus rhamnosus*

1. Introduction
Glucose can be used for the production of lactic acid. *Lactobacillus delbrueckii* and *Lactobacillus rhamnose* contain lactic acid-producing microorganisms. More complex enzymes would be able to shape higher populations of *Lactobacillus delbrueckii*. Axelsson (1998) states that glucokinase is a complex enzyme that plays a role in the production of lactic acid. This enzyme will transform 1 mole of glucose into 2 moles of lactic acid in order to maximize the production of lactic acid in the silo environment via a high population of lactic acid bacteria (Filya, 2000). As a consequence, lactic acid concentration is higher. Meanwhile, several homofermentative lactic acid bacteria are unable to produce pure L (+) lactic acid. As the only product utilizing heterofermentative species, *Lactobacillus rhamnosus* is supplied with L (+) lactic acid. Under anaerobic conditions, these species generate ethanol along with L (+) lactic acid. L.rhamnosus has been produced by chemical mutagenesis for this function. This phenomenon in batch fermentation has been investigated. The benefit of this approach is that the only product produced is pure L (+) lactic acid production. (Niju et al, 2004)
Lactic acid has two isomeric forms, namely L (+) or D (-) lactic acid. Lactic acid can be produced by chemical synthesis and fermentation processes. The chemical synthesis process will produce lactic acid, which is a mixture of two isomers. The fermentation process will produce specific lactic acid, namely L (+) lactic acid or D (-) lactic acid (Narayan et al., 2004). Lactic acid can be applied commercially in the environmentally friendly polymer industry (biodegradable polymer) (Litchfield, 2009).

Glucose, a monosaccharide sugar, is one of the essential carbohydrates used as the primary source of energy in the body. Glucose has a precursor for the synthesis of all other carbohydrates in the body (Murray R. K. et al., 2003). The substrate used to produce lactic acid has obtained from pure glucose. Meanwhile, the bacteria used to convert glucose into lactic acid were using lactic acid bacteria Lactobacillus delbrueckii and Lactobacillus rhamnosus. Research on the conversion of glucose to L-lactic acid from lactic acid bacteria has been formulating into three formulations. First, what is the growth curve profile of Lactobacillus delbrueckii and Lactobacillus rhamnosus bacteria? Second, how the influence of pH, agitation, and glucose concentration on the formation of L-lactic acid, third, which bacteria produce the highest lactic acid. Based on the existing problem formulations, this study has three objectives, (1) to determine the profile of the growth curve of the bacteria Lactobacillus delbrueckii and Lactobacillus rhamnosus; (2) to assess the effect of pH, agitation, and glucose concentration on the formation of L-lactic acid; (3) to find out what bacteria produce the highest lactic acid.

The glucose medium used is pure glucose. Glucose is converted to lactic acid by lactic acid bacteria, namely, Lactobacillus delbrueckii and Lactobacillus rhamnosus. The two bacteria separately fermented on glucose medium. Both bacteria were grown first on the media to see the profile of the growth curve so that the optimum incubation time has obtained. After that, the fermentation process itself with variations in the type of bacteria and glucose concentration has been giving. The resulting lactic acid can be determined through analysis using the titration method and analysis with a UV VIS spectrophotometer.

2. Materials and Methods

2.1 Preparation of glucose stock solution 1000 mg / l
0.1 grams Weighed of anhydrous glucose. They have dissolved with aquadest in beaker glass. Transferred to a 1000 ml volumetric flask and diluted to mark the limit then shaken until homogeneous.

2.2 Preparation of a standard glucose solution
10 ml of glucose stock solution 1000 mg / l has taken. The solution has to put into a 100 ml volumetric flask. And added aquadest to the boundary mark then shaken until homogeneous. To obtain a standard solution of glucose 10 mg / l. The solution is taken 1 to 22 ml successively. Each has to put in a 10 ml volumetric flask. Then added aquadest to mark the limit and shaken until homogeneous. So that the glucose standard solution has obtained with a concentration of 220 mg / l.

2.3 Standard curve for glucose solution
Ten test tubes have been prepare. Each was filled with 2 ml glucose standard solution with a concentration of 10-220 mg / l. Then 2 ml was taken and put in a test tube. Then added 2 ml of DNS reagent. The test tube has covered with aluminum foil and then heated in a boiling water bath for 5-10 minutes. After that, it cooled then measured the absorbance with a wavelength of λ 540 nm.

2.4 DNS reagent
For a solution of 1 weigh 1 g of DNS and 1 g of NaOH, dissolve it in 40 ml of H2O. For a solution of 2 weigh 20 gr Potassium Tatrat 20 gr, 0.2 g phenol, and 0.05 g Sodium Metabisulfite, dissolve in 40ml H2O. Then mix the two solutions in a 100 ml volumetric flask and dilute to the mark.

2.5 Regeneration of bacteria
Lactobacillus delbrueckii and Lactobacillus rhamnosus were rubbed on the medium to slant MRS media then grown at 30-40°C for seven days. The bacteria have inoculated in 100 ml of pre-culture media on a 250 ml Erlenmeyer volume and then incubated in an Incubator shaker (150 rpm) at 30-40°C for 12 hours.

2.6 Growth Curve
Enter each of the 25 ml of regenerated bacteria into four Erlenmeyer, each containing 225 ml of media. They have then incubated in an Incubator shaker (150rpm) 30-40°C for 48 hours. Take a 10 ml sample every 3 hours to observe the OD value, glucose levels, and lactic acid levels.

2.7 Fermentation Process
Fermentation has carried out under optimum conditions at temperature (30-40°C), pH 5-6, 18 hours, 1-5% glucose concentration using MRS medium, and BHM medium.

2.8 Lactic Acid Analysis
Lactic acid analysis has carried out by the titration method. The quantitative test of Lactic Acid levels was carried out alkaliometry with the titration method. First, enter 1 ml of the fermented results, and dilute to 10 ml with distilled water in 250 ml Erlenmeyer, add three drops of 1% pp indicator solution. Then proceed with titration with 0.25x10^-2 N NaOH solution until pink color changes. Acid content is calculated as lactic acid by the formula:

% Lactic Acid = ((VxN) NaOH x BM A. Lactate x 100%) / (Material Weight (g) x 1000)

2.9 Analyze glucose levels
Two ml of the sample taken every 3 hours have added to 2 ml of DNS reagent. Then it is heated in boiling water, and the homogeneous solution will change color to become more concentrated. After that, the samples here analyzed using a UV-VIS spectrophotometric instrument with a wavelength of 540 nm.

3. Result and discussing
In this study, we compared the performance of Lactobacillus delbrueckii and Lactobacillus rhamnosus bacteria in the glucose conversion process to lactic acid, which has been applying as the primary raw material for making biodegradable plastics. Glucose, a monosaccharide sugar, is one of the essential carbohydrates used as the primary source of energy in the body. (Murray R. K. et al., 2003). Lactic acid (2-hydroxypropanoic acid (CH3-COH-COOH), also known as milk acid) is an essential chemical compound in several biochemical processes. Lactic acid can be applied commercially in the environmentally friendly polymer industry (biodegradable polymer) (Litchfield, 2009).

Lactic acid can have produced from starch and cellulose, which are processed by fermentation. Lactic acid form, starch must first be broken down into simpler molecules, namely glucose, through the hydrolysis process. Furthermore, glucose will be broken down into lactic acid through fermentation by lactic acid bacteria (Rintis, 2010). In this study, we used two types of bacteria, namely, Lactobacillus delbrueckii and Lactobacillus rhamnosus.

Lactobacillus delbrueckii bacteria are homofermentative bacteria that can produce lactic acid in large enough quantities (Jin Bo et al., 2005). Lactobacillus delbrueckii generally grows in temperatures ranging from 30 to 40 degrees Celsius (Siegrist, 1997). Lactobacillus delbrueckii does not increase below 15°C or above 45°C (Siegrist, 1997). Lactobacillus delbrueckii has been categorizing as a facultative anaerobic. Which means it is mainly aerobic, but can also grow anaerobically.

The isolation of Lactobacillus rhamnosus has carried out using MRS de Man Rogosa and Sharpe selective media, and the best temperature for the growth of these bacteria was 42°C. Lactobacillus rhamnosus bacteria will react positively to some medium. The medium is rhamnose, cellobiose, maltose, glucose, mannitol, salicin, trehalose, sorbitol, xylose, and sucrose. And react negatively to arabinose and rallmose media (GIBBS et al., 1966).
3.2.1 Regeneration of Bacteria on Media

The bacteria to have used are regenerated to refresh the bacteria so that the bacteria can adapt to the new media (obtained by young bacterial cells). De Man Rogosa and Sharpe (MRS) has used as a medium for bacterial culture. The MRS made consists of yeast extract, which has been operating as a source of protein, peptone as a source of nitrogen and beef extract K2HPO4, ammonium citrate, 2 grams of glucose, sodium acetate. 3H2O, MgSO4.7H2O, and MnSO4.4H2O as a source of other nutrients. The addition of distilled water serves to dissolve the composition of the MRS. The next step after the MRS was cold, and bacteria were planted and then incubated in a shaker incubator for 24 hours with a temperature of 30-40°C. The incubator shaker functions to circulate the air that is in the Erlenmeyer so that there is better oxygen circulation for bacterial growth.

The purpose of incubation itself is to condition the environment at the optimum temperature for bacterial development so that it can have been that the bacteria are developing correctly. Observations have made after 24 hours, on the control MRS media, it was clear, which showed that there was no bacterial growth in the nutrient broth, while in the MRS media the inoculated bacteria looked cloudy, indicating the presence of bacterial growth in the nutrient broth (Mulyadi, 2013).

3.2.2 Growth Curves

The development of the growth curve aims to determine the optimum time for Lactobacillus delbrueckii and Lactobacillus rhamnosus to produce lactic acid. After knowing the optimal growth time for Lactobacillus delbrueckii and Lactobacillus rhamnosus bacteria when the growth curve has been carrying out to obtain the highest lactic acid, this time has used as the best time for lactic acid production. The growth of Lactobacillus delbrueckii and Lactobacillus rhamnosus on fermentation has indicated by the increasingly concentrated solution that is fermented. The growth curves of Lactobacillus delbrueckii and Lactobacillus rhamnosus have been measure from the absorbance analysis, lactic acid levels, and glucose levels, which were taken every 3 hours and plotted to determine the growth profile of these bacteria. The lag phase occurs at 0 to 3 hours. Log phase at 3 to 21 hours, and stationary phase at 24 to 42 hours.

Figure 3.1 Growth Curves of Lactobacillus delbrueckii and Lactobacillus rhamnosus in MRS Media

![Growth Curves](image_url)
Figure 3.2 Growth Curves of Lactobacillus delbrueckii and Lactobacillus rhamnosus in BHM Media
((Lactobacillus rhamnose, Lactobacillus delbrueckii)

The growth curve for MRS media and glucose experiences a lag phase or an adaptation phase at 0 to 3 hours. In this phase, there is an adjustment process for both Lactobacillus delbrueckii and Lactobacillus rhamnosus bacteria to their environment (Pratiwi, 2008; Dwidjoseputro, 1994). The log phase or exponential phase on MRS media occurs at 3 to 21 hours, while glucose occurs at 3 to 18 hours. In this phase, the cells are in a state of balanced growth. During this phase, the population doubles at regular time intervals. The number of bacterial colonies will continue to increase, along with the speed of cell metabolic activity (Pratiwi, 2008; Dwidjoseputro, 1994).

The stationary phase or fixed phase on MRS media occurred at 24 to 42 hours, while in BHM, media occurred at 21 to 24 hours. In this phase, there was a competition between bacteria to obtain nutrients from the media to stay alive. Some bacteria die while others grow and divide so that the number of living bacterial cells remains. (Pratiwi, 2008; Dwidjoseputro, 1994). The death phase or dead phase only occurs in bacteria grown with BHM media. This phase occurs at 24 to 42 hours. In this phase, bacterial cells will die faster than the formation of new cells. The death rate has accelerated exponentially (Lee, J, 1983).

3.2.3 Analysis of Lactic Acid

The quantitative test of lactic acid levels was carried out alkalimetry with the titration method. The total titrated acid is the amount of lactic acid formed during the fermentation process. Where in fermentation, glucose will have converted into lactate (lactic acid). Determination of the Total Titrated Acid (TAT) in fermented glucose has been carrying out to determine the acid content in the product. The resulting acid content will significantly affect the growth of lactic acid bacteria in fermentation (Reskia, 2013).

Production of lactic acid by Lactobacillus delbrueckii and Lactobacillus rhamnosus on MRS media (Figure 4.3) during fermentation increased at 3 to 21 hours then decreased at 21 to 42 hours. This production has following the number of bacteria produced in each hour—the time at the time of fermentation. During fermentation, the number of bacteria also increases and decreases at the same time. So it can be concluded that if the number of bacteria has increased, the lactic acid levels will also increase, and vice versa.
Figure 3.3 Lactic Acid Level Curves of Lactobacillus delbrueckii and Lactobacillus rhamnosus in MRS Media (■ Lactobacillus rhamnose, Lactobacillus delbrueckii)

Figure 3.4 Curves of Lactobacillus delbrueckii and Lactobacillus rhamnosus in BHM Media (■ Lactobacillus rhamnose, Lactobacillus delbrueckii)

While the production of lactic acid by Lactobacillus delbrueckii and Lactobacillus rhamnosus on BHM media (Figure 4.4) during fermentation increased at 3 to 24 hours then decreased at 24 to 42 hours. These conditions following the number of bacteria produced at any time during fermentation. During fermentation, the number of bacteria also increases and decreases at the same time. So it can be concluded that if the number of bacteria has increased, the lactic acid levels will also increase, and vice versa.

Based on the levels of lactic acid produced using both media, it can have been seeing that in the graph, Lactobacillus delbrueckii bacteria give a better performance in making lactic acid compared to Lactobacillus rhamnosus. The Lactobacillus delbrueckii bacteria are homofermentative bacteria in which glucose has fermented to produce lactic acid as the only product. Meanwhile, the Lactobacillus
rhamnosus bacteria are heterofermentative bacteria in which glucose is fermented, besides producing lactic acid, it also has other compounds, namely ethanol, acetic acid, and CO2.

### 3.2.4 Analysis of Glucose

Analysis of glucose levels has carried out using spectrophotometry and by adding a DNS reagent to the sample. The consideration of using this method is because it is easy to do, and the results obtained are more satisfying for measuring reducing sugar. Besides, DNS reagents have commonly been using to measure reducing sugars produced by microbes because of their high degree of accuracy so that they can be applied to even small levels of sugar. However, this method has a drawback, namely that the DNS gene will experience instability if there is direct contact with light so that the storage of DNS reagents must have avoided direct contact with sunlight (Kodri, 2013). The results of measuring glucose levels in each sample using the DNS (Dinitrosalicylic Acid) method has shown in Figure 4.5 and Figure 4.6.

Figure 3.5 Curves of Lactobacillus delbrueckii and Lactobacillus rhamnosus glucose levels in MRS Media (Lactobacillus rhamnose, Lactobacillus delbrueckii)

Figure 3.6 Glucose Curves of Lactobacillus delbrueckii and Lactobacillus rhamnosus in BHM Media (■ Lactobacillus rhamnose, ■ Lactobacillus delbrueckii)
The high glucose consumption in the early hours was at the 3rd hour (Figure 4.5 and Figure 4.6) using both MRS and BHM media. This figure shows that glucose at the beginning of fermentation has been operating for the growth of Lactobacillus delbrueckii and Lactobacillus rhamnosus. Glucose is a source of energy and carbon source for Lactobacillus delbrueckii and Lactobacillus rhamnosus. As an energy source, glucose is fermented by Lactobacillus delbrueckii and Lactobacillus rhamnosus to become lactic acid. Therefore, every hour the glucose level decreases because glucose is consumed continuously for bacterial growth and the production of lactic acid.

### 3.2.5 Relationship between Glucose Levels and Lactic Acid

Lactic acid can have produced from starch and cellulose, which are processed by fermentation. The process lactic acid form, starch must first be broken down into simpler molecules, namely glucose, through the hydrolysis process. Furthermore, glucose will be broken down into lactic acid through fermentation by lactic acid bacteria (Rintis, 2010).

Consumption of glucose has been using for the growth of Lactobacillus delbrueckii and Lactobacillus rhamnosus. Glucose is a source of energy and carbon source for Lactobacillus delbrueckii and Lactobacillus rhamnosus. As an energy source, glucose has fermented by Lactobacillus delbrueckii and Lactobacillus rhamnosus to become lactic acid so that there is a relationship between glucose consumption and the levels of lactic acid that has formed. A decrease in glucose levels accompanies an increase in lactic acid levels at any time. This condition is due to the high glucose consumption in the early hours, namely at 3 hours. Glucose at the beginning of fermentation has been using for the growth of Lactobacillus delbrueckii and Lactobacillus rhamnosus as an energy source and carbon source. As an energy source, glucose is fermented by Lactobacillus delbrueckii and Lactobacillus rhamnosus to become lactic acid. Therefore, every hour the glucose level decreases because glucose is consumed continuously for bacterial growth and the production of lactic acid so that the increase in lactic acid levels is inversely related to the decrease in glucose levels consumed.

The maximum amount of lactic acid produced in 60 hours (full time) of fermentation wherein the lactic acid content reaches the highest amount is 45 g / L. these results are similar to those studied by Xiaomeng (2013). Glucose has widely consumed, and only 0.85 g / L remains after fermentation. Therefore, the conversion of corn waste pre-treated with LHW (Liquid Hot Water) to lactic acid with R. oryzae is very efficient. From the figure below, it can have to be seen that using MRS Media can produce a higher% Lactic Acid. MRS media has high nitrogen and glucose content. The addition of nutrients and a higher concentration of nutrient mass generally has a positive effect on lactic acid production. MRS media contains Yeast Extract (0.5%), Urea (0.5%), dipotassium hydrogen phosphate (0.1%), Sodium Acetate (0.5%), Magnesium sulfate (0.03%), and glucose. Higher concentrations or better nitrogen sources can improve reactor performance. Yeast extract has been considering to be an essential nutrient for Lactobacillus for efficient lactic acid production. Especially supplementation with MRS media, the total nitrogen increases so that both the acceleration of the bioconversion rate and the production of lactic acid is doubled (Vijayakumar, 2007). This condition is what causes fermentation using MRS media to produce higher levels of lactic acid than using BHM media.

![Figure 3.7 Curve Between Lactic Acid Levels and Glucose Levels of Lactobacillus delbrueckii in MRS Media](image-url)
Lactic acid bacteria fermentation has been divided into two groups of fermentation, homofermentative and heterofermentative. This bacteria produced lactic acid. Lactobacillus delbrueckii has a homofermentative group (Karimah et al., 2011). Lactobacillus rhamnosus is heterofermentative, which ferments lactose with the final result of fermentation to produce lactic acid and acetic acid in a ratio of 2:3 and ethanol in large quantities (Charteris et al., 2002).

### 3.2.6 Profile of Lactic Acid Formation with Bacteria and Glucose Concentration as Variables

In this study, the manufacture of lactic acid has carried out with the help of the bacteria Lactobacillus delbrueckii and Lactobacillus rhamnosus, which were able to convert glucose into lactic acid. Before
the fermentation process has been carrying out, it is necessary to regenerate the bacteria Lactobacillus delbrueckii and Lactobacillus rhamnosus, which has been using as a starter in the fermentation has carried out. The starter was Lactobacillus delbrueckii and Lactobacillus rhamnosus, which were grown in MRS media and BHM media. The inoculum of Lactobacillus delbrueckii and Lactobacillus rhamnosus has been taken from the regeneration of the bacteria on MRS slant agar media. The starter has made so that the bacteria Lactobacillus delbrueckii and Lactobacillus rhamnosus could adapt to the new growing media.

The fermentation process in this study used MRS media and BHM media. MRS media has chosen because it contains yeast extract (0.5%), urea (0.5%), dipotassium hydrogen phosphate (0.1%), sodium acetate (0.5%), magnesium sulfate (0.03%), and glucose with different concentrations, where glucose has converted to lactic acid. Besides, MRS media is a suitable medium for the formation of lactic acid. Meanwhile, BHM media has chosen because it is ideal for glucose growth.

The fermentation carried out in this study used a variable type of bacteria and glucose concentration. The MRS and BHM media used were 90%, each of which has added with the bacteria Lactobacillus delbrueckii and Lactobacillus rhamnosus cultures each of 10% of the media. The glucose concentration variables added to the MRS media were 40, 50, 60, 70, and 80%. While the variable concentration of glucose added to the BHM medium was 10%, 20%, 30%, 40%, 50%, and 60%.

From Figure 3.12, the addition of glucose concentrations of 40, 50, 60, 70, and 80% into the MRS media to be fermented. At each addition of glucose, there was an increase up to the addition of 60% glucose concentration, the number of bacteria Lactobacillus delbrueckii and Lactobacillus rhamnosus produced increased as well. In this figure, the highest number of bacteria has added to the glucose concentration by 60%. This result is consistent with the statement of Jene (2004) that glucose concentration has a significant effect on the amount of lactic acid produced. Glucose is used as a precursor and initial source of energy by lactic acid bacteria before fermenting other carbohydrates. However, a high enough glucose concentration (up to 70%) can inhibit microbial growth, and sugar has generally used as a preservation technique (Estiasih and Ahmadi, 2009). what causes after the addition of glucose with levels of 70% - 80%, the bacteria cannot survive. So, the addition of glucose that is too high can inhibit microbial growth, and this is not economically beneficial because pure glucose is expensive. Lactic acid is a cheap product (Hofvendahl and Hagerdal, 2000).
Figure 3.13 Curve of Bacterial Numbers to Glucose Concentration in Lactobacillus delbrueckii and Lactobacillus rhamnosus on BHM Media (■ Lactobacillus rhamnose, ■ Lactobacillus delbrueckii)

Figure 3.13 shows the addition of glucose concentrations of 10%, 20%, 30%, 40%, 50%, and 60% into the BHM media to be fermented. At each addition of glucose into this medium, the number of bacteria produced has increased then decreased. In this study using BHM media, glucose concentration did not affect the number of bacteria produced. The addition of glucose concentrations between 10% to 30% in media using Lactobacillus delbrueckii bacteria and 10% to 20% in media using Lactobacillus rhamnosus bacteria, glucose is capable of sufficient bacterial growth. So that the addition of glucose with a concentration of 10% to 30% for Lactobacillus delbrueckii and a concentration of 10% to 20% for Lactobacillus rhamnosus bacteria will be more economical because it does not affect the decrease in the growth of lactic acid bacteria.

From Figure 3.14, the glucose concentration of 40-80% is added to the MRS media to be fermented. With each addition of glucose, there is an increase up to 60% glucose levels, the resulting lactic acid levels also increase. In this figure, the highest lactic acid content is in the addition of glucose concentration by 60%. This result is consistent with the statement of Jene (2004) that glucose concentration has a significant effect on the amount of lactic acid produced, and glucose is used as a precursor and initial source of energy by lactic acid bacteria before fermenting other carbohydrates. However, a high enough glucose concentration (up to 70%) can inhibit microbial growth, and sugar has generally used as a preservation technique (Estiasih and Ahmadi, 2009). the addition of glucose 70% - 80%, the levels of lactic acid have decreased because the number of lactic acid bacteria has also reduced. So, the addition of glucose that is too high can inhibit microbial growth, and this is not economically beneficial because pure glucose is expensive. Lactic acid is a cheap product (Hofvendahl and Hagerdal, 2000).
Figure 3.14 Curves of Lactic Acid Levels to Glucose Concentration with Lactobacillus delbrueckii and Lactobacillus rhamnosus on MRS Media (■ Lactobacillus rhamnose, ■ Lactobacillus delbrueckii)

Figure 3.15 Curve of Lactic Acid Levels to Glucose Concentration with Lactobacillus delbrueckii and Lactobacillus rhamnosus on BHM Media (■ Lactobacillus rhamnose, ■ Lactobacillus delbrueckii)

From Figure 3.15, glucose concentrations are added by 10%, 20%, 30%, 40%, 50%, and 60% into the BHM medium to be fermented. With each addition of glucose has increased, the resulting levels of lactic acid also increase. However, there was an extreme increase in levels of 30% -40% and 50% -60% using Lactobacillus delbrueckii bacteria and 20% -30% and 40% -50% with Lactobacillus rhamnosus bacteria. In this figure, the highest lactic acid content is in the addition of glucose concentration by 60%. This result is consistent with the statement of Jene (2004) that glucose concentration has a significant effect on the amount of lactic acid produced. Glucose is used as a precursor and initial source of energy by lactic acid bacteria before fermenting other carbohydrates. So, the more glucose has been added, the supply of nutrients for lactic acid bacteria is more so that more lactic acid bacteria live and triggers a higher production of lactic acid as well.

An increase in glucose levels accompanies an increase in lactic acid levels at any time. The level of glucose added to the media has also increased. Glucose at the beginning of fermentation has been using for the growth of Lactobacillus delbrueckii and Lactobacillus rhamnosus as an energy source and carbon source. As an energy source, glucose is fermented by Lactobacillus delbrueckii and Lactobacillus rhamnosus to become lactic acid. Therefore, high glucose levels will help the growth of bacteria for the production of lactic acid so that the increase in lactic acid levels is directly proportional to the rise in glucose levels consumed.

From Figure 3.16, it can have been seeing that using MRS Media can produce a higher% Lactic Acid. MRS media has high nitrogen and glucose content. The addition of nutrients and a higher concentration of nutrient mass generally has a positive effect on lactic acid production. MRS media contains Yeast Extract (0.5%), Urea (0.5%), dipotassium hydrogen phosphate (0.1%), Sodium Acetate (0.5%), Magnesium sulfate (0.03%), and glucose.
Figure 3.16 Curve of Lactic Acid Levels to the Concentration of Glucose Remaining in Lactobacillus delbrueckii Bacteria on MRS Media

Figure 3.17 Curve of Lactic Acid Levels to the Concentration of Glucose Remaining in Lactobacillus rhamnosus Bacteria on MRS Media

Figure 3.18 Curve of Lactic Acid Levels to the Concentration of Glucose Remaining in Lactobacillus delbrueckii Bacteria on BHM media
Higher concentrations or better nitrogen sources can improve reactor performance. Yeast extract has been considered to be an essential nutrient for lactobacillus for efficient lactic acid production. Especially supplementation with MRS media, total nitrogen increased so that both the acceleration of the bioconversion rate and the production of lactic acid were doubled (Vijayakumar, 2007). fermentation using MRS media to produce higher levels of lactic acid than using BHM media.

Figure 3.19 Curve of Lactic Acid Levels to the Concentration of Glucose Remaining in Lactobacillus rhamnosus Bacteria on BHM media

Homofermentative bacteria are capable of producing lactic acid from glucose as much as 85-90%. While heterofermentative bacteria only have about 50% lactic acid from glucose (Surono, 2004). Homofermentation only produces lactic acid as the end product of glucose metabolism, and in this process, the Embden-Meyerhoff-Parnas pathway has been using. In the heterofermentative process of lactic acid, carbon dioxide, and ethanol have been producing in equal molar amounts through the phosphocytolase way (Hofvendahl and Haegerdal, 2000). the bacteria Lactobacillus delbrueckii to produce higher levels of lactic acid compared to Lactobacillus rhamnosus. Besides, we tried the addition of 70-80% glucose levels in MRS media because of up to 60% glucose levels. The number of bacteria still increased. However, a high enough glucose concentration (up to 70%) can inhibit microbial growth, and sugar has generally used as a preservation technique (Estiasih and Ahmadi, 2009). the addition of glucose 70% -80%, the levels of lactic acid have decreased because the number of lactic acid bacteria has also reduced. So, the addition of glucose that is too high can inhibit microbial growth, and this is not economically beneficial because pure glucose is expensive. Lactic acid is a cheap product (Hofvendahl and Hagerdal, 2000).

**Conclusion**

From the research on "Comparing the Performance of Lactobacillus delbrueckii and Lactobacillus rhamnosus Bacteria on the Conversion of Glucose to Lactic Acid," it can be concluded that: In the growth curve profile, the optimum time obtained by the bacteria Lactobacillus delbrueckii and Lactobacillus rhamnosus on the conversion of glucose to lactic acid occurs in the log phase, which is 21 hours. The addition of a high enough glucose concentration (> 60%) to fermentation can inhibit microbial growth. Lactobacillus delbrueckii can produce higher levels of lactic acid in the two media used. Compared to Lactobacillus rhamnosus because Lactobacillus delbrueckii is homofermentative, which can produce 2-lactic acid. It has been recommending that research on the manufacture of lactic acid from glucose conversion be developed further with glucose from lignocellulose degradation. It is advisable to carry out the process of producing lactic acid on a large scale so that it can be used directly in the industry.
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