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Production of Poly Lactic Glycolic Acid from Solid Waste of Palm Starch Industry for Applications in the Field of Medical Biomaterials

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ABSTRACT

Palm starch industry bring out solid waste that contain high total cellulose (cellulose and hemicellulose), that is 82,03%. Cellulose from solid waste of palm starch industry can be used in lactic acid production through hydrolysis and fermentation processes. Enzymatic hydrolysis and fermentation processes will work efficient and effectively when run simultaneously in one reactor, known as Simultaneous Sacharificatian and Fermentation (SSF). This research aimed to find out the most effective pH and quantity of inoculum in SSF process. This research was using substrate from solid waste of palm starch industry that treated by delignification using NaOH 7% and then saccharificated and fermented simultaneously using enzyme from Trichodermareesei for saccharification process, and Lactobacillus delbrueckii bacteria FNCC 0045 for lactic acid fermentation. The research was held by pH variation of 4, 5, 6 and 7, while quantity of inoculum were 5, 10, 15, 20 and 25% respectively. The incubation was at 46°C for 96 hour. The result showed that optimal pH was 6 and 25% inoculum was the best. Production of PLGA films was started with the polymerization reaction between lactic acid and glycolic acid at various ratios of monomer (wt%) = 95:5 and PLGA :PVA at various ratio (wt%)= 3:2, 3:3, 3:4, 3:5.The solution of PLGA in chloroform was added with PVA solution (as the film forming agents) and aloe vera gel (as an anti-microbial agents) subsequently, and then stirred with a magnetic stirrer for about 10 minutes until homogeneous. The homogeneous PLGA solution thereafter poured on the mold (glass plate). The results showed that the greater the amount of poly vinyl alcohol (ratio 3:5 % by weight), the greater the tensile strength of the film, but the lower elongation..

Keywords: *solid waste of palm starch industry; lactic acid; SSF;PLGA*

1. INTRODUCTION

Almost all over the archipelago, palm tree can grow well. Palm stem from various regions in Indonesia were sent to several centers of starch sugar industry. In District Tulung, Klaten regency, Central Java, there are 25 large-scale producers of palm starch. Of these activities generate solid waste starch sugar industry about 2 tons / day. Sentra starch sugar industry in the region Tulung not manage industrial solid waste starch aren well due to limited understanding of the craftsmen. Currently the waste is simply dumped in the river banks around the population, resulting in water and environmental pollution due to waste raises stink. The craftsmen starch aren not realize that solid waste generated from the processing of starch sugar still has economic value.

Based on previous research (Purnavita and Sriyana, 2011), industrial solid waste containing cellulose starch aren total (cellulose and hemicellulose) is high, ie 82.03%. Cellulose content in solid waste starch sugar industry can be utilized to lactic acid through hydrolysis and fermentation process. Enzymatic hydrolysis and fermentation process will be extremely efficient and effective if implemented on an ongoing basis which is often known as Simultaneous Sacharificatian and Fermentation (SSF) (Sreenath et al, 2001). Making the lactic acid from the cellulose with SSF process, stages of saccharification and fermentation of cellulosic substrates produced glucose take place simultaneously in one step and in one reactor. In the method of SSF, cellulose substrates simultaneously degraded into oligosaccharides and hydrolyzed to glucose by the enzyme activity, then the glucose produced directly fermented by Lactobacillus bacteria into lactic acid. According to Wang et al (2010), the optimum temperature production of lactic acid from corn straw with SSF method is 45oC.

According to Litchfield (2009), the lactic acid produced by bacterial activity homofermentatif will produce a single product, ie only pure lactic acid without other metabolic products, such as organic acids, alcohols, aldehydes, ketones, and carbon dioxide. Homofermentatif bacteria used to produce pure lactic acid include Lactococcus lactis and Lactobacillus delbrueckii. Results of lactic acid from glucose by homofermentatif in general can achieve a yield of 90% or higher.

Based on previous research, industrial solid waste palm starch can be hydrolyzed to extract termites and continued fermentation with Lactobacillus casei and managed to produce lactic acid although the levels are still low at 0.91 g / L (Purnavita et al., 2013). Hydrolysis and fermentation processes that do still separate so it takes a long process and inefficient. Lactic acid levels obtained from these studies is still low, this is because the types of microbes that are used when in contact with air so that the results will be heterofermentatif impure lactic acid fermentation, but also generated other organic acids.

Lactic acid is an organic acid that can be polymerized into poly lactic acid or glycolic copolymerization of poly lactic acid (Poly Lactic Acid Glycolic, PLGA) (Erbetta et. al, 2012; Gentile et. al, 2014). PLGA is a hydrophobic biopolymers are biodegradable and biocompatible properties of the most well compared to poly lactic acid (Poly Lactic Acid, PLA) for applications as biomaterials in the medical field. According to Parida et. al (2011), PVA is a synthetic polymer that is hydrophilic, strong, good film-forming ability, and biodegradable so it can be applied in the medical field.

Filming of biomaterial PLGA with the addition of hydrophilic polymer (PVA) and additive (aloe gel) using a solution casting method as biomaterials for biomedical applications and characterization. The addition of PVA aims to improve the mechanical strength and film forming ability, while aloe vera gel as an antimicrobial additive and stimulate tissue regeneration. As far as literature searches were conducted, no conducting research filming of PLGA biomaterials with modified PVA and aloe vera gel.

2. RESEARCH METHODS

2.1 Materials

Industrial solid waste palm starch is derived from starch sugar industry center in the village of Daleman, District Tulung, Klaten regency, Central Java. Cellulase enzymes used in SSF process is derived from Trichoderma reesei ATCC 26 921 with Sigma-Aldrich brand USA. Lactic acid bacteria used were Lactobacillus delbrueckii culture FNCC-0045, obtained from the Center for Food and Nutrition Studies UGM. Media de Man-Rugosa-Sharpe (MRS) from Merck. Yeast Extract Powder of HIMEDIA RM 027.

2.2 Substrate preparation

The process of Pretreatment of the solid waste starch sugar industry refers to previous studies (Purnavita et al, 2013).

2.3 Bacteria Preparation and Making Medium

Stock culture of Lactobacillus delbrueckii FNCC-0045 was inoculated on MRS-agar medium in the form of so upright, incubated at 37 °C for 3x24 hours. Inoculum made in MRS broth, incubated at 37°C for 3x24 hours.

Medium SSF are used to contain the substrate 20 g / L, yeast extract 30 g / L, NaOH 1.25 g / L, K_2HPO_4 0.2 g / L, KH_2PO_4 0.2 g / L, $MgSO_4.7H_2O$ 0.6 g / L, $MnSO_4.H_2O$ 0,03g / L, $FeSO_4.7H_2O$ 0.03 g / L (Yoon, 1997).

2.4 Sacharification Simulthaneous Fermentation

Media were sterilized coupled with cellulase enzymes as many as 45 units / 2 g susbstrat. Cellulase enzymes before being added to the first substrate was dissolved in deionized water. The variable in this study is the media pH variation which is 4, 5, 6, 7 and the amount of inoculum, ie 5, 10, 15, 20 and 25%. SSF process is carried out at a temperature of 46 ° C for 96 hours.

3. RESULT AND DISCUSSIN

3.1 Lactic Acid Production Process

The process of making lactic acid from starch aren industrial solid waste was conducted using simultaneous saccharification and fermentation (SSF) at 45oC and 96 hours. Saccharification industrial solid waste starch into glucose sugar performed enzymatically using cellulase enzymes of Trichoderma reesei and then simultaneously continue the process of fermentation of glucose to lactic acid by the bacteria Lactobacillus delbrueckii. The lactic acid produced is tested quantitatively with total acid method.

Variations in pH and the amount of inoculum made in this study to determine the initial pH of the most effective media in the SSF process, and determine the amount of inoculum in units of percent that gives the best results. Variations in pH chosen is 4, 5, 6 and 7, with variations in the amount of inoculum 5, 10, 15, 20 and 25%. Results lactic acid levels and yields are shown in Table 1.

The initial process of SSF is the breakdown of cellulose in the substrate to glucose by cellulase enzyme activity. Glucose is produced from the process of saccharification by cellulase enzymes will be used by the bacteria Lactobacillus delbrueckii to produce lactic acid.

Table 1. Parameters of the study (lactic acid content and yield)% on a wide range of pH and the amount of inoculum

Riset Parameters	pH	The amount of inoculum (%)				
		5	10	15	20	25
Lactic acid level (g/L)	4	4,0146	4,8189	4,5593	4,5593	4,8291
	5	4,0146	4,5593	4,8291	4,8291	4,8240
	6	3,7343	8,4261	7,9342	8,1846	8,9138
	7	5,8931	6,6164	8,6720	8,4261	7,6882
Yield (%)	4	20,0728	24,0943	22,7965	22,7965	24,1454
	5	20,0728	22,7965	24,1454	24,1454	24,1202
	6	18,6712	42,1305	39,6707	40,9229	44,5690
	7	29,4654	33,0819	39,6707	42,1305	38,4412

The results of this study are not consistent with research Yoon (1997), which uses the same bacteria but different enzyme, the enzyme Cytolase CL, showing the optimal pH is 5, while the SSF performed at pH 4 and 5 in this study gives the results of lactic acid lower than at pH 6 and 7. the cellulase enzyme activity which is the initial stage of the process requires initial conditions corresponding to the characteristics of the enzyme. According Adney and Baker (2008), the optimal conditions for the activity of cellulase from Trichoderma reesei is at pH 4.3 to 4.5. This study did not follow the saccharification and fermentation process at each unit of time, so that the only result obtained at the end of the process. This causes can not be known a great time at the commencement of the lactic acid fermentation. Optimal conditions for the growth of Lactobacillus delbrueckii is 6.2 to 6.5; then even at pH 4 or pH 5 more glucose resulting from saccharification process, but the activity of lactic acid bacteria is not high because the pH is not an optimal condition.

If linked to the amount of inoculum, the results showed that in general, at pH 6 and pH 7 lactic acid levels increase with increasing percent inoculum was added to the fermentation medium. This is consistent with research Chairunnisa, et al (2006) contained in Purnavita, et al (2013) which states that the higher doses of lactic acid bacterial starter culture led to increasingly sharp increase in the rate of lactic acid production.

Results of statistical tests to compare the levels of lactic acid between pH in the same amount of inoculum showed that there were significant differences in all groups the amount of inoculum. Catching results it appears that the results of levels of lactic acid at pH 4 and 5 showed a significant difference in levels of lactic acid at pH 6 and 7, while the levels of lactic acid at pH 6 and 7 did not show any statistical difference.

Lactic acid levels produced in this study between 4.0 to 8.9 g / L, much higher than the study Purnavita, et al (2013) which produces lactic acid levels below 1 g / L, even with up to 30 percent inoculum %. This study was conducted using Simultaneous saccharification and Fermentation (SSF), in contrast with research Purnavita, et al (2013) using the method of fermentation-rise, which separates the hydrolysis of cellulose to glucose fermentation. SSF method in this study proved effective to produce lactic acid with high enough levels, because the cellulose substrate stage saccharification and fermentation of glucose results saccharification carried out simultaneously in one reactor (Sreenath et al, 2001). Saccharification stage in the study conducted by cellulase enzymes of Trichoderma reseei, while the fermentation of glucose to lactic acid made by the bacteria Lactobacillus delbrueckii. The process of saccharification by cellulase enzymes purely takes about 10 hours, and then the glucose produced will soon be converted into lactic acid levels will increase along with increasing time (Yoon, 1997).

Yoon research results produce lactic acid with a concentration of about 12 g / L in 80 hours of fermentation. This study gives lower yields, ie the maximum concentration of 8.9 g / L. This result was expected, given the cellulose substrate used in this study is the dregs of palm that still contain lignin compound and complex carbohydrates other than cellulose. Research Yoon (1997) used a substrate in the form of commercially pure cellulose, so sakarifikasi process can occur more quickly because there is no dirt compound. The result is lactic acid levels obtained from this study showed that the highest levels obtained in SSF conditions with pH 6 and the amount of inoculum 25 %. Based on the parameters that yield solid waste starch sugar industry could potentially be used for the production of lactic acid as it can provide the lactic acid yield solid waste substrate of 44.6%.

3.2 Biofilm Characteristic

Characteristics Tensile Strength and Elongation Film

Determination of tensile strength (TS) or tensile strength is the maximum voltage that occurs in the film that can withstand the load while being stretched or pulled before damage occurs. Strength (strength) is the nature of the materials most besar meterial ditentukan oleh voltage capable loose before it breaks (failure). Based on the research results as shown in Table 2, for a ratio of PLGA with PVA (Poly Vinyl Alcohol) which vary the composition of PLGA same constituent monomers turned out to give a different tensile strength. The greater the amount of PVA added value that the lower tensile strength. The highest tensile strength obtained at a ratio of 3 PLGA than PVA than 5, this shows that the greater number of PVA than PLGA will provide greater tensile strength, Long breaking (elongation at break) or the process of elongation is the maximum length change during a stretch until interrupted film sample.

Table 2. Relationship PLGA Ratio: PVA Against Tensile Strength

Ratio LA : GA	Ratio PLGA : PVA	Tensile Strength (N/mm^2)
95% : 5%	3 : 2	2,2106
	3 : 3	2,1686
	3 : 4	2,4297
	3 : 5	2,9451

In general, the greater the amount of PLGA than PVA will give a percentage strain (elongation). The addition of PVA on the filming of the PLGA polymers are made from raw material sugar starch industrial solid waste serve to improve the film forming on the making of the film with the casting method, but the results showed that the addition of PVA will lower percentage strain (elongation). The greater the amount of PLGA than PVA will yield a value of percent elongation of the film is getting bigger.

Table 3. Relationship PLGA Ratio: PVA Against Tensile Elongation

Ratio LA : GA	Ratio PLGA : PVA	Percentage Strain (Elongation)
95% : 5%	3 : 2	46,5261
	3 : 3	39,6867
	3 : 4	42,0270
	3 : 5	41,8287

Characteristics of FTIR

In Figure 1 appears peaks at wave numbers 675-995 cm-1 that may indicate the presence of C-H groups alkene usually appears in wave numbers 675-995 and 3010-3095 cm-1. Appeared peaks at wave numbers 690-900 cm-1 that may indicate the presence of C-H group Aromatic rings that usually appear on the wave numbers 690-900 and 3010-3100 cm-1. It also appears a peak at the wave number 1050-1300 cm-1 that may indicate the presence of C-O group Alcohol / ether / carboxylic acid / ester usually appears in wave numbers. Based on the description above it can be concluded that the polymer PLGA properly formed.

Figure 1. FTIR Film PLGA : PVA

4. CONCLUSION

a The production of lactic acid with a substrate of solid waste starch sugar industry and methods Simultaneous saccharification and Fermentation (SSF) using cellulase enzymes of Trichoderma reseei and Lactobacillus delbrueckii occurs most effectively at pH 6 media and the amount of inoculum 25%. Optimal lactic acid levels of 8.9 g / L and the yield of lactic acid to the substrate of solid waste starch sugar industry amounted to 44.6%.

b 2. The film with the highest tensile strength and low percent elongation obtained on the composition of the PLGA (95%: 5%) with a ratio of PLGA: PVA = 3: 5.

c 3. Test results showed appear FTIR peak at the wave number 1050-1300 cm-1 that may indicate the presence of C-O group Alcohol / ether / carboxylic acid / ester.

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