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HYDROLYSIS OF SAGO (METROXYLON SAGO ROTTB.) PITH POWDER BY SULFURIC ACID AND ENZYME AND FERMENTATION OF ITS HYDROLYZATE BY *PICHIA STIPITIS* CBS 5773, *SACCHAROMYCES CEREVISIAE* D1/P3GI, AND *ZYMOMONAS MOBILIS* FNCC 0056 INTO BIOETHANOL

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Abstract

The purpose of this study was to determine hydolysis efficiency of sago pith powder and fermentation efficiency by P. Stipitis CBS 5773, S. cerevisiae D1/P3GI, and Z. mobilis FNCC 0056.. This research was experimentally and consist of hydrolisis process sago pith powder and fermentation of its hydrolyzate. Sago pith powder has content 77,5% starch, 4,63% cellulose, 4,86% hemicelluose, lignin 3,07%, dan water 10,12%. In gelatination process in temperature at 120˚C, the yield of sugar concentration 0,62% and dextrose equivalent (DE) 0,89% obtained from the size of 100 mesh. Sago gelatine hydolized by sulfuric acid acid, α-amylase, hemicellulase, cellulase, dan amyloglukosidase produce sugar concentration 53,28% and DE 68,52%. At fermentation process in sugar concentration 5% with content of glucose 4.17 %, fermentation by Pichia stipitis, Saccharomyces cerevisiae, and Zymomonas mobilis provide ethanol fermentation efficiency 23,5%, 37,65%, dan 32,13% respectively. At fermentation process with sugar concentration 10% with content of glucose 8,28 %, fermentation by Pichia stipitis, Saccharomyces cerevisiae, and Zymomonas mobilis yielding ethanol fermentation efficiency 9,30%, 50,85% and 20,65% respectively.

Key words**:** Hydrolysis, Sago Pith Powder, fermentation*, Pichia stipitis CBS 5773, Saccharomyces cerevisiae D1/P3GI, Zymomonas mobilis FNCC 0056*

INTRODUCTION

Indonesia currently has a high dependence on fossil fuels and has at least three serious problems that the depletion of petroleum reserves, the instability of fuel prices due to high demand and rising greenhouse gas (CO2) from burning fossil fuels so that Indonesia needs alternative and renewable energy sources. On the other side of Indonesia as a tropical country that has many resources, especially carbohydrates and cellulose from agricultural waste. Especially for Eastern Indonesia has vast forest sago has great potential for renewable energy sources and energy independence.

Bio-ethanol is ethanol produced by fermentation by various microorganisms (yeasts and bacteria) [1]. Currently ethanol is used as 'fuel additive' (fuel mixture), it can even replace non-renewable fuel conventionally.

One of substrate which is potential and environmentally friendly to produce ethanol is a sago plant. Although Sago need long age harvest is about 6 years but can produce starch in large quantities. Sago is Indonesia plant native, can grow on various soil types, such as: dry soil, clay, swamp or soil flooded. Sago palm acreage in Indonesia is very wide which is about 1.128 million ha [2]. Seedling density of about 1480 trees / ha when the crop yield and 125-140 trees / year [3]. (Each of the sago tree containing an average of 200-400 kg sago starch, so can obtain 30-60 tonnes of sago starch / ha [4]. (Bintoro, 2003). Trunk of sago pith without peel has weight an average 850 kg with starch content 29 percent, water content 50 percent and fibers ranged 21% [5]. Overall utilization of sago for ethanol production is perceived to be more effective because of the stem consists of the sago pith which is containing starch and fiber. Sago starch content about 84.7 to 85.9% with the percentage of amylose 27 % and amylopectin 73%[2], while the sago fiber composed of cellulose and hemicellulose, when hydrolyzed

completely into simple sugars, especially glucose[2].

In this study uses Sago (Sago Metroxylon Rottb.) obtained from Kibin, Banten. Sago is processed (self-preparative) sago flour. Based on proximate test, the pith of sago contain of starch about 77.50%,and fiber 12:56%, water 10.12%. Total amount of sugar contained in the pith flour equal to the amount of carbohydrate. Total sugars of pith flour in size 50 mesh is equal to 60.47% (w / w), it meansthat every 1 g of the pith flour contained 0.604 g of carbohydrates. While the total sugar contained in the pith flour 100 mesh size is equal to 77.76% (w / w) means that in every 1 g of the pith of flour contained 0.778 g of carbohydrates. With high carbohydrate content means sago is very potential as a major source of sugar for ethanol fermentation.

Several groups of microorganisms have the ability to produce ethanol from carbohydrates through the fermentation process. Some microorganisms such as Pichia stipitis can ferment xylosa . Saccharomyces cerevisiae, and Z. mobilis has the ability to ferment hexose (C6) [6]. While Z.mobilis is a type of bacteria that can produce ethanol from hexose sugars and fermentation capabilities faster than S. cerevisiae [7].

On lignocellulose biomass is necessary to pre-treatment such as desizing of the pith flour and temperature of gelatinization. Optimum temperature of the pre-treatment can increase the acid and enzyme -hydrolysis which is expected to produce sugars from cellulose then fermented into ethanol. Concentration of ethanol produced is influenced by the type and concentration of sugar. Because microorganisms have different preferences and tolerances to concentration of sugar.

Pre-treatment. Pre-treatment of lignoselulose needs to be done prior to enzymatic or acid hydrolysis. Pretreatment aims to solve, dissolve, hydrolyze and separate components of cellulose, hemicellulose, and lignin [8]. (Saha, 2003). Various methods for biomass pretreatment methods such lignoselulosik autohydrolisis,treatment with organic solvent, thermo mechanical; milling, refining, cutting, extortion, acid treatment; acid solution (H2SO4, HCl), concentrated acids (H2SO4, HCl),treatment with alkali; Sodium hydroxide, ammonia, hydrogen and peroxide alkaline [8]. The use of acid for the initial treatment materials can lower the cost for enzyme.

Gelatinization and Saccharification. The conversion process of starch, cellulose, and hemicellulose into sugars through the stages of gelatinization and hydrolysis. Stages of gelatinization facilitate the action of the enzyme hydrolyzes the substrate. Gelatinization is influenced by temperature, substrate type and concentration of the substrate itself. The smaller of gelatin concentration the higher of reducing sugar content. Because acid is difficult to diffuse to the high starch concentrations. Hydrolysis is the decomposition of polysaccharides into monosakarida. Hydrolysis carried out by crushing the raw material of sago mixed with water, acids or enzymes such as termamyl, at pH 6.5 and heated at a temperature of 90oC. Further saccharification process using dextrozim for 24 hours at pH 4.5 and temperature 60 ° C[9] (Bujang, Ishizakhi, and Goh Ping Yau, 2006).

Hydrolysis can be performed using acids, enzymes, or mixtures of acids and enzymes. The acid used can be either concentrated acid or dilute acid. Acid will break down the starch molecules randomly and sugars produced mostly reducing sugars. At the optimum conditions for starch molecules will produce 88% glucose. However, in practice, using the acid hydrolysis of starch to glucose would only result in the value of DE (Dextrose Equivalent) 55%. If the value of DE (Dextrose Equivalent) above 55%, it will produce furfural compounds and acid hydroximethyl levulinat which can inhibit the fermentation process.

Hydrolysis of polysaccharides into monosaccharides using enzyme carbohydrase, namely: α-amylase, hemiselulase, cellulase, and amiloglukosidase. The amount of enzyme amiloglukosidase at full dose (0.56 μ l / g) were used to hydrolyze starch (100%), whereas the amount of cellulase enzymes on the full dose (0.83 μ l / g) were used to hydrolyze pure cellulose Hydrolysis of starch and cellulose will produce glucose and additional products in the form of sugar that is hydroximethyl furfural derivatives. Hemicellulose hydrolysis would produce two types of sugar, pentose and

hexose. This research will study the influence of particle size reduction in pith flour of sago, gelatinization temperature, hydrolysis effectiveness of combination of acid and enzyme in hydrolysis and fermentation capabilities of *Pichia stipitis Sacchromyces cerevisiae and Zymomonas mobilis* in hydrolyzate results of sulfuric acid hydrolysis 6 M followed by the enzyme in hydrolysis.

MATERIAL AND METHOD

Pre-treatment and Hydrolysis. The research was carried out experimentally in the laboratory and conducted in three stages: (1) Pre-treatment: consists of optimization of particle size reduction (50 mesh and 100 mesh) of pith flour of sago, optimization of temperature in gelatinization which are 90°C, 100°C, 110°C, dan 120°C C for 20 min in 1 atm pressure (2). Acid and enzymatic hydrolysis in Gelatin pith flour. In the hydrolyzate of the pre treatment that produces the highest reducing sugar content was added 6 M sulfuric acid, hereinafter incubated for 1 hour at 120^oC in this hydrolizate (I) was . measured the concentration of reducing sugar and Dextrose equivalent (DE). Once hydrolyzed by 6 M sulfuric acid hidrolisate (I) is cooled to 25^0C and adjusted to pH 6.0, then α-amylase enzyme is added as much as 0.17 ml / g (volume enzyme/ g substrate), incubated at 104^{0C} for 60 minutes with a pressure of 1 atm (HII). The hydrolyzate II measured the concentration of reducing sugar and dextrose equivalent (DE). Hydrolyzate II is heated at a temperature of 121^0C with a pressure of 1 atm for 10 minutes. Then added with hemiselulase as much as 1/3 dose. Hydrolysed further incubated at 55°C with agitation of 150 rpm for 270 minutes (hydrolyzate III) hydrolyzate III had cooled 25^0C. Then the pH was adjusted to 4.8. After that cellulase enzymes are added as much as 0.55 ml / g and amyloglucosidase 0.37 ul g (volume enzyme / g substrate). Subsequently incubated at 60^0C for 48 h with agitation 130 rpm. At this stage, concentration of reducing sugar and DE is measured.(Gerhartz,1990).

Fermentation by Pichia stipitis CBS 5773, Saccharomyces cerevisiae D1/P3GI, and Zymomonas mobilis FNCC0056 a single culture to ferment sugars hydrolizate pith of sago starch hydrolyzate.

The reducing sugar content measurement method done by DNS. Sago as much as 15 g gelatin dissolved in distilled water until the volume is 25 ml. The next sample is introduced into a centrifuge tube and centrifuged at 3500 rpm for 20 minutes. Clear sample solution of 1 ml pipetted into a test tube inserted. Then into the test tube was added 3 ml of DNS (3.5 Dinitrosalicylic acyd). After that, samples were homogenized with a vortex and heated in a boiling water bath for 5 minutes. After five minutes of heated, then cooled in an ice bath. After the absorbance was measured using a spectrophotometer at a wavelength of 550 nm. Calculation of Dextrose Equivalent (DE) using the formulaDextrose Equivalent (DE) = reducing sugar concentration of the sample (%) x 100%/ Total sugar concentration (%).

Preparation of Substrate Fermentation and fermentation. The hydrolyzate which is having highest DE (Dextrose Equivalent) and reducing sugars content. then adjusted to 5% and 10% ofreducing sugar content.. Each hydrolyzate is added with fermentation medium containing (per liter): yeast extract 4g, KH_2PO_4 2g, $(NH4)_2SO_4$ 3g, MgSO4.7H2O1g, and pepton 3, 6 g (Sanchez et al., 2002). pH was adjusted to pH 7. For fermentation by P. stipitis CBS 5773 and S. Cerevisiae D1/P3GI pH medium was adjust to pH 5, and medium for Z. mobilis FNCC 0056 pH adjusted to ph 7. Fermentation medium were sterilized. For fermentation every cultures (Pichia stipitis CBS 5773 S. Cerevisiae D1/P3GI and Z. mobilis FNCC 0056) as much as 10% put into a fermentation substrate, which is containing hydrolyzate with concentration sugar are 5% and 10%. Then cultures in medium shake-incubated at 30^o C for 72 h with agitation 150 rpm. During incubation, samples were taken at 0, 6, 12, 18, 24, 30, 36, 48, 60, and 72 hours for measurement of ethanol and reducing sugar content.

RESULTS AND DISCUSSIONS

Optimization of desizing of partikel of pith flour and temperature incubation in gelatinized Pith flour used in this study had a starch content 77.5%, cellulose 4.63%,

hemicellulose 4.86%, lignin 3.07% and water 10.12%. Prior to the hydrolysis step, the substrate made gelatinization. Gelatinization is a mechanism of entry of water into the starch granules so as to facilitate the action of the enzyme hydrolyzes the substrate. Gelatinization is influenced by temperature, substrate type and concentration of the substrate itself [10]. Treatment with temperature 90˚C, 100˚C, 110˚C, and 120˚C of the pith flour of in 50 and 100 mesh resulted a reducing sugar concentrations were as follows.

Table 1. Duncan's multiple range test analysis of Concentration of reducing sugar in size of pith flour 50 mesh and 100 mesh.

Temp of Gelatinization (˚C)	Cons. Of Red. sugar (%) in 50 mesh flour	Cons. Of Red.sugar (%) in 100 mesh
90	0,28a	0,25a
100	0,35b	0,27a
110	0,42c	0,40b
120	0,53d	0,62c

From the results of Duncan's multiple range test analysis can be seen that the increase in gelatinization temperature, the greater the concentration of sugar produced. The results of starch gelatinization of sago pith 50 mesh at 120˚C is a reducing sugar concentration of 0.53%. While on 100 mesh at 120˚C obtained 0.62%. Concentration of sugar produced from the pith of sago in 100 mesh size larger than 50 mesh. 100 mesh size, mean grain size of pith starch particles is 175µ. While the 50 mesh size, mean grain size of pith starch particles is 350µ.. The smaller a particle, the greater the absorption area [11].

Table 2. Duncan's multiple range test analysis of Dextrose equivalent (DE) at various temp. on 50 mesh and 100 mesh

Temp of Gel. (˚C)	Dextrose equivalent (DE) (%) in size 50 mesh	Dextrose equivalent (DE) (%) in size 100 mesh
90	0,51a	0,31a
100	0,57b	0,32b
110	0,69c	0,52c
120	0,85d	0,89d

Grain flour 100 mesh has a smaller particle size will absorb more heat and water, therefore the more that comes out of the starch amylose so that the concentration of sugar produced more than 50 mesh size. Dextrose equivalent (DE) indicates the number of starch polymers that have been cut into glucose molecules are much simpler. Dextrose equivalent can be obtained from the ratio of the concentration of sugar samples with a total sugar concentration.

From the analysis of Duncan's multiple range test t is known that increasing the temperature of gelatinization, the greater the dextrose equivalent (DE) is generated. From starch gelatinization of sago pith flour 50 mesh obtained the highest DE 0.85% at 120˚C and DE 89% at 100 mesh. This is due to the high temperature will facilitate the breaking of carbon-hydrogen bond, so that more polymer is cut starch into glucose[12]. Gelatin then is hydrolyzed. chemically and enzymatically, Chemical hydrolysis using sulfuric acid 6M and the pH was adjusted to 2.

Tabel 3: Conc. of red. sugar and (DE) of substrat is hydrolyzed by sulfuric acid 6 M and amylase, hemicellulase, cellulase and amyloglucosidase

Treatment of Hydrolysis	Cons. Of red. sugar.	DE (%)
Sulfuric acid 6 M	22,6	28,63
α-amylase	33,02	42,47
Hemicellulase	33,94	43,65
Cellulase and Amyloglucosidase	53,28	68,52

DE = *dextrose equivalent*

Concentration of reducing sugar produced in the pith flour of sago is hydrolyzed by sulfuric acid is measured by DNS method. Hydrolysis using sulfuric acid 6M produce concentration of reducing sugars 22.26% and DE2 8.63% . On the acid hydrolysis, acid breaks the bond glycosidic pith flour of sago at random but consecutive to the smallest molecule called glucose. However, the results of a reducing sugar and DE obtained indicates that not all pith flour of sago can be hydrolyzed by acid. This is in accordance with the statement [13] that the conversion of starch using acid will only generate a maximum of DE 55%.

Hydrolysis by enzymes

Hydrolysates I generated at acid hydrolysis using sulfuric acid, and then hydrolyzed by the enzyme α-amylase with doses 0,17 ul/g, which lasted for 60 minutes at a temperature of 104ºC and pH 6.0, hydrolysis using α-amylase enzyme, obtained hydrolyzate II ith reducing

sugars obtained amounted to 33.02%, and DE 42.47%. Increased concentrations of reducing sugars due to the breakdown amylose and amylopectin in starch by α-amylase which does not occur at optimum in the acid hydrolysis. α-amylase is an endo-enzyme that can break the bond of alpha-(1,4) glycosidic randomly. Break down of amylose by α-amylase into maltose and maltotriosa that occur at random. Then form of glucose and maltose as the final result. While working of α-amylase on the amylopectin would produce glucose, maltose, and various types of oligosaccharide [15]. Hydrolysates II produced by α-amylase then hydrolyzed by hemicellulase. Hydrolysis by hemicellulase for 27^0 minutes at a temperature of 55ºC and pH 6.0. Concentrations of enzyme were added at 1/3 of the recommended enzyme concentration is equal to 0.001 g / g substrate. Hydrolysis by hemicellulase obtained hydrolyzate III.

The main purpose hemicellulase, fibers which is containing hemicellulase will produce simple sugar such as hexose and pentose. The use of hemicellulase is able to raise of a reducing sugar concentration and DE, reducing sugar from 33.02% to 33.94% and DE from 42.47% to 43.65%.. The use of hemicellulase not only able to contribute of increase in reducing sugar concentration of the hydrolysis of hemicellulose but also helps the action of the enzyme cellulase..

Hydrolysis by cellulase enzymes and Amyloglucosidase. The addition of cellulase done because in the previous stage, the hydrolysis of cellulose was not optimal. While the addition amiloglukosidase made to hydrolyze dextrins obtained from the previous stage so as to produce glucose. Provision of both types of enzyme was carried out simultaneously as cellulase and amiloglukosidase have synergistic activity.Hydrolysis by cellulase enzymes and amiloglukosidase (saccharification) was held at a temperature of 60 º C and pH 4.8. DX dextrozyme enzyme dosage was 0.37 ml / g (dose of 2/3), while the number of Celluclast 1.5 L is inserted is 0.55 ml / g (dose of 2/3). Saccharification carried out for 48 hours. Hydrolysis using dextrozyme DX and Celluclast 1.5 L produces a reducing sugar concentration [% (w / w)] 53.28% and DE 68.52% after 48 hours of incubation.

Cellulase enzymes play a role at the beginning of the process is made starch granules more open so that amyloglucosidase a chance to hydrolyze starch granules inside which is have higher of sensitivity towards amiloglukosidase activity[15]. Reducing sugar concentration increased, this is due to cellulase enzymes break down cellulose into selobiosa. Selobiosa is then split into glucose. In addition, DX dextrozyme breaking glycosidic bond α-1, 4 and α-1, 6 produce monomers of glucose. Bond of α-1 ,4-glycosidic found in maltose and maltotriosa which is the result of a process of amylose and amylopectin liquefaction while bonds α-1 ,6-glycosidic which is the dextrins present in the process liquefaction of amylopectin.

At this stage of the hydrolysis of the resulting concentration of DE for 68.52%. This is consistent with the statement Langlois and Dale (1940) in Tjokroadikoesoemo [16] that the hydrolysis process using a combination of acid and enzymes can increase the value of DE. Initially carried out by acid hydrolysis process to DE 55%, then uses the enzyme hydrolysis followed by amilolitik to DE 61-65%. In this study, DE values greater than 65% can be caused by the addition of the cellulolytic enzyme hydrolysis process, the cellulase and hemicellulase so that the amount of glucose produced more. Hydrolyzates of sugar which is the result of hydrolysis, then set the concentration to 10% and 5%. HPLC analysis of the results obtained that 10% of sugar hydrolizate consists of glucose 8.28% and maltotetraosa 1.72%. Sugar concentration of 5% consisted of glucose 4.17% and maltotetraosa 0.83%. Sugar is then fermented by *P.stipitis, S.cerevisiae, and Z.mobilis* a single culture.

Efficiency fermentation of *Pichia stipitis, Zymomonas mobilis and Sacchromyces cerevisiae* in fermentation of reducing sugars of hydrolyzate of pith of sago starch. Concentration of sugar and ethanol at the end of the process of ethanol fermentation of hydrolyzate sugar by *Pichia stipitis*. Hydrolyzate fermentation medium sugar

concentration of 5%, containing glucose 4,17% glucose and maltotetraosa 0.83%. While the concentration of sugar hydrolyzate medium 10% containing glucose 8.28% glucose and 1.76% maltotetrosa.

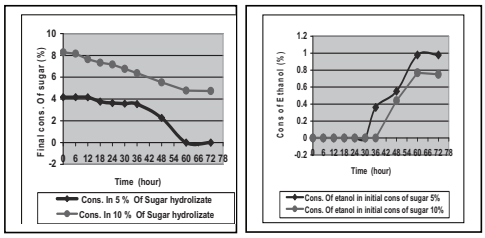

Fig 1. Reducing sugar and ethanol concentration changes during fermentation hydrolyzate of pith of sago starch by *P.stipitis*

During the fermentation process takes place, the concentration of sugar hydrolyzate 5% containing glucose 4,17% is used up by *P.stipitis* at the 60 hours. While the initial sugar hydrolyzate concentration 10%, after 72 hours as much as 8.28% glucose used by P.stipitis and leaving glucose 4.73%, this means only 3.55% used by *P.stipitis*. This suggests that *P.stipitis* less tolerant to sugar concentration 10%. Sugar is used as a nutrient and for ethanol fermentation. On medium hydrolyzate sugar 5% containing glucose 4,17% produces ethanol only 0.98% which began to form after 36 hours, meaning that up to 36 hours glucose used for nutrition. From the fermentation is well known efficiency of glucose fermentation by Pichia stipitis only 23.5%, while according to theory, 100% sugar when fermented will produce 50% ethanol [12].

Concentration of ethanol in the fermentation by *P.stipitis* sugar in a medium of sugar hydrolyzate 10% containing glucose 8.28%, produces ethanol only as much as 0.77% for 72 hours and ethanol is formed at 48, it means up to 36 hours, glucose used as a nutrient. Concentration of ethanol produced in the hydrolyzate 10% less than the ethanol produced in the sugar hydrolyzate 5%. thus the efficiency of fermentation by *P.stipitis* on glucose medium containing 8.28% only 9.30%.

Efficiency of fermentation by *Pichia stipitis* is very low, it is presumed *P.stipitis* less able to ferment hexose sugars. The best ability to ferment pentose sugars. More glucose is used to increase the cell biomass. Also *P.stipitis* have a low tolerance to high sugar concentration. This can be seen in 10% glucose medium, *P.stipitis* lag phase longer than the medium of 5%.

Concentration of reducing sugar and ethanol at the end of the process of ethanol fermentation of hydrolyzate sugar by *Saccharomyces cerevisiae*

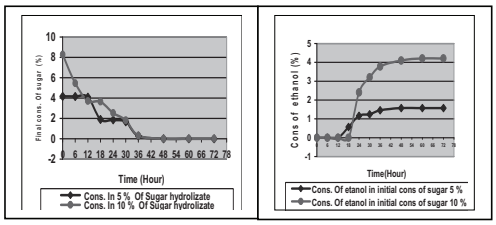

Fig 2. Reducing sugar and ethanol concentration changes during fermentation hydrolyzate of pith of sago starch by S. cerevisiae

Hydrolyzate sugar concentration both at 5% and 10%, during the fermentation process, glucose is used up by the S.cerevisiae for 36 and 48 hours. This shows S.cerevisiae has the ability to ferment hexose sugars, particularly glucose very well. S.cerevisiae has invertase enzyme that acts to convert glucose into ethanol[17] . Sugar medium consisting of glucose 4.17%, fermentation by S.cerevisiae for 72 hours produced 1.57% ethanol and ethanol have started to form after 18 hours. Efficiency of fermentation in sugar hydrolyzate 5% by S.cerevisiae as much as 37.6%. While the concentration of ethanol produced by S.cerevisiae on fermentation of sugar hydrolyzate 10% in medium containing glucose 8.28%, producing ethanol as much as 4.21% for 72 hours. Fermentation efficiency of 49.2% reaching almost 50%, this corresponds to the theory that 100% of sugar when fermented will produce 50% ethanol [12].

Concentration of reducing sugar and ethanol at the end of the process of ethanol fermentation of hydrolyzate sugar by *Zymomonas mobilis*.

During the fermentation process, hydrolyzate sugar concentration 5% containing glucose

4.17% is used up by Z.mobilis after 36 hours and the ethanol yield of 1.34%.

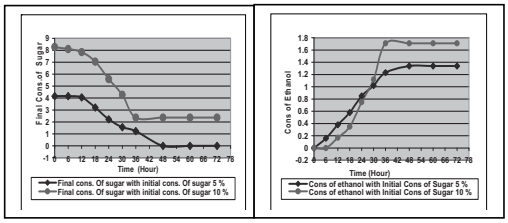

Fig 3. Reducing sugar and ethanol concentration changes during fermentation hydrolyzate of pith of sago starch by *Z.mobilis.*

Glucose is used for cell growth and ethanol fermentation substrate. Whereas in sugar hydrolysates 10% containing glucose 8, 28% still remaining glucose. .37%. This is due to bacterial cells die after 36 hours, while ethanol produced as much as 1.34% for 72 hours, the ethanol begins to form after 6 hours. Based on amount of ethanol that is formed is known that the sugar hydrolyzate 5% fermentation efficiency Z.mobilis reachs 32.13% while on the sugar hydrolyzate 10%, ethanol fermentation efficiency reach 20.65%. According to[18]. Z.mobilis has a good ability to ferment glucose, the results are even greater than the S.cerevisiae due to the formation of ethanol by the Entner Doudoroff. In this study the fermentation of sugars hydrolyzate b Z.mobilis obtained a little amount of ethanol, because the medium pH dropped to 3:37 is so low that bacterial cell death occurred.

CONCLUSIONS

1. Desizing of pith of sago starch particles in the size of 100 mesh, and gelatinization temperature at 120 ˚ C, can increase the reducing sugar and dextrose equivalent (DE).
2. Hydrolysis by acids and enzymes can be applied in synergy so as to increase the activity on the hydrolysis of lignocellulosic materials.
3. Efficiency of fermentation in the sugar hydrolyzate sugar concentration of 5% containing of glucose 4.17% *Pichia stipitis, Sacchromyces cerevisiae* and *Zymomonas mobilis*, respectively are 23.5%, 37.65% and 32.13%. While in the hydrolyzate sugar concentration10%of sugar with glucose content 8.28%, fermentation efficiency of *Pichia stipitis, Sacchromyces cerevisiae* and *Zymomonas mobilis* respectively are 9.3%, 50.85% and 20.65%.

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