

Production Of Bioethanol From Corncob And Sugarcane Bagasse With Hydrolysis Process Using *Aspergillus niger* and *Trichoderma viride*

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Abstract : Bioethanol is an alternative fuels derived from biomass that is more environmentally friendly and renewable. Corncob and sugarcane bagasse are a potential agricultural wastes used as raw material for bioethanol because it has a high carbohydrate content and abundant availability. The efforts to increase bioethanol production performed by enzymatic hydrolysis method using fungus cultures of *Aspergillus niger* and *Trichoderma viride*. This study aims to determine the best combination of the addition of *A. niger* and *T. viride* cultures on corncob and sugarcane bagasse raw materials which produces optimal bioethanol production with high substrate efficiency and low residual sugars content. The results showed that the use of corncob raw materials with the addition of *Aspergillus niger* culture is the best treatment combination that produces ethanol at 282,3 ml/kg with 90,35% substrate efficiency. While the bagasse raw material, the addition of *Trichoderma viride* culture is the best treatment combination that produces ethanol at 299,2 ml/kg with 90,95% substrate efficiency.

Keyword: Bioethanol, hydrolysis, *Aspergillus niger*, *Trichoderma viride*, fermentation

I. Introduction

The use of fossil fuels contributes for a very large air pollution, which transportation sector contributes about 80% of CO₂ emissions in atmosphere. Bioethanol (C₂H₅OH) is an ethanol produced from biomass or natural raw materials through fermentation of sugar with the help of microorganisms, such as yeast. Bioethanol is a fuel that is more environmentally friendly and renewable.

Some species of plants have been investigated for bioethanol production, such as cassava, sugarcane, corn and waste products of agriculture (Muthuvelayudham and Viruthagiri, 2007). Potential agricultural waste used as bioethanol raw materials are corncob and sugarcane bagasse. Corncob has a high carbohydrate content and is able to produce ethanol at 0,358 liter/kg dry mass. While bagasse can produce ethanol at 0,279 liter/kg dry mass (Kuhad and Singh, 1993).

Bioethanol production process generally consists of four steps, namely the process of material preparation or pretreatment, hydrolysis, fermentation and distillation. Hydrolysis process is an important step because it determines the amount of glucose produced as a substrate to be fermented into ethanol. Hydrolysis process can be carried out with an acid catalyst, the catalyst combination of acid and enzymes, as well as the catalyst enzyme and enzyme (Muthuvelayudham and Viruthagiri, 2007; Patel et al., 2007).

Bioethanol production is still not optimal. One is because the saccharification process only lasts for destruction by the heating process, so that the amount of sugars produced is not optimal. The efforts to increase the production of bioethanol can be done by using a cellulolytic fungus culture in the process of hydrolysis of lignocellulose by enzymatic hydrolysis method. Lignocellulose degrading enzymes are cellulase and hemicellulase. Some groups are often utilized cellulolytic fungus in lignocellulose degradation are *Aspergillus niger* and *Trichoderma viride* (Prasad et al., 2009; Tribak et al., 2002). Both of this fungus consist of three classes of cellulase enzymes that endoglucanases, cellobiohydrolases and β -glucosidases (Juwaied et al., 2011). Cellulase enzyme activity produced by *A. niger* and *T. viride* is higher compared with the other fungus, namely *A. nidulans*, *A. terreus*, *A. wentii*, *T. reesei*, *T. koniginii* and *T. lignorum* (Juwaied et al., 2011). The purpose of this research is to determine the best combination of the addition of culture *Aspergillus niger* and *Trichoderma viride* on corncob and sugarcane bagasse raw materials which produces optimal bioethanol production and high substrate efficiency with low residual sugar content.

II. Materials And Methods

2.1 Tools and materials

Materials that used in the bioethanol production are corncob, sugarcane bagasse, fungus cultures (*Aspergillus niger* and *Trichoderma viride*) and yeast (*Saccharomyces cerevisiae*). Chemical materials in the

pretreatment and hydrolysis process using NaOCl 0,15 N, NaOH 2 N, HCl 0,5 N, Na₂CO₃ 2 N, aquadest, ammonium sulfate, peptone, PDA and YMGP. The tools used in the production and analysis of bioethanol are; oven, autoclaf, analytical balance, pH meter type 900 IT WalkLAB, haemocytometer, Picnometer, Gas Chromatography (GC) and Rotary vacuum evaporator type Heidolph.

2.2 Research methods

This research using randomized complete design with eight treatments and three replications. Observed factors are the type of raw materials and the addition of culture *Aspergillus niger* and *Trichoderma viride* on the hydrolysis process. The first factor is the type of bioethanol raw materials, namely corncob and sugarcane bagasse. The second factor is the addition of fungus culture in the hydrolysis process, namely: Without fungus addition, with the addition of *Aspergillus niger* culture, with the addition of *Trichoderma viride* culture and with the addition of combination cultures of *Aspergillus niger* + *Trichoderma viride*.

2.3 Pretreatment

First step delignification corncob and sugarcane bagasse materials that had been ground to a size of 60 mesh. The first is done by soaking the delignification of 500 grams of each materials using NaOCl 0,15 N at room temperature for five hours with a ratio of 1: 8 (materials: solvent). Second delignification using NaOH 2 N at room temperature for 24 hours with a ratio of 1: 4 (materials: solvent). The result are corncob and sugarcane bagasse powder.

2.4 Acid and enzymatic hydrolysis

Hydrolysis method using acid and enzymatic hydrolysis. The first step, delignified corncob and sugarcane bagasse from each of the added water with weight ratio of 1: 5 (powder: water). HCl 0,5 N was added to adjust the acidic conditions (pH ±4,5). Substrate hydrolyzed by heating at a temperature of 115 ° C for 60 minutes using autoclave. The next sep, the hydrolyzate was added 10% (v/v) culture of *Aspergillus niger* and *Trichoderma viride* in accordance with each treatment. For the combination treatment of *Aspergillus niger* + *Trichoderma viride*, the amount added is respectively 5% (v/v) from each *Aspergillus niger* and *Trichoderma viride*. Hydrolysis performed at room temperature for 72 hours. Furthermore, the results of hydrolysis neutralized with Na₂CO₃ 2 N until pH ±6.

2.5 Preparation of fungus and yeast culture

Aspergillus niger and *Trichoderma viride* cultured in PDA (Potato Dextrose Agar) were made in test tubes and grown in an incubator at a temperature of 28 °C for 7 days. *Saccharomyces cerevisiae* yeast isolates rejuvenated on PDA and incubated for 2 days. Furthermore, isolates were grown again in 50 ml of YMGP media consisting of yeast extract 5 g/l, malt 5 g/l, glucose 10 g/l and peptone 5 g/l in 200 ml erlenmeyer. Incubation was performed at 30 °C for 24 hours.

2.6 Fermentation and distillation

Fermentation takes place in anaerobic conditions. Substrate hydrolysis of each materials added to 1 g/l peptone and 4 g/l ammonium sulfate as nutrients. Then added yeast *Saccharomyces cerevisiae* culture as much as 10% (v / v). The fermentation process lasts for seven days. During the fermentation process the pH value, total sugar and ethanol content were analyze. After eight days, filtered fermented, then distillation to separate the ethanol from the water fraction of the substrate fermentation at a temperature 78-80 °C using Rotary vacuum evaporator.

2.7 Analysis of fiber composition

Analysis of fiber include cellulose, hemicellulose and lignin content, performed on the material before and after delignification process (pretreatment), and substrate hydrolysis residues.

2.8 Analysis of reducing sugars and total sugars content

Reducing sugar content was analyzed by the method of DNS, while the total sugar content was analyzed by the method phenol. Reducing sugar analysis performed on substrate hydrolysis. While total sugar analysis performed during the fermentation process every 24 hours.

2.9 Analysis of ethanol content

Analysis of ethanol content (% v/v) was performed on the fermentation process, measured using Gas Chromatography (GC) and picnometer. Observations made during the ethanol fermentation process every 24 hours until the end of the fermentation process.

2.10 Ethanol yield

Ethanol extracts obtained from the separation by distillation which has been separated from the water. Measured volume of ethanol obtained from 500 grams of dry mass.

2.11 Substrate efficiency

Substrate efficiency is the percentage concentration of substrate (total sugars) are consumed for the production of the initial substrate concentration used in the production. The amount of substrate consumed is the difference between the initial concentration of total sugar (So) with the concentration of sugar at the end (S) of production processes. Substrate efficiency is calculated by formulas:

$$\text{Substrate efficiency (\%)} = \frac{S_o - S}{S_o} \times 100\%$$

2.12 Data analysis

Data processing were analyzed using analysis of variance (F test 5%). If there is a significant effect on treatments, then continued using LSD test to detect differences between treatments.

III. Results And Discussion

3.1 Fiber composition of materials during pretreatment and hydrolysis processes

Based on Table 1, shows a decrease in the lignin content of corncob material, before delignification 12,74% to 2,99% after delignification. Lignin content in the sugarcane bagasse materials also decreased, before delignification 22,87% to 1,50% after delignification. Data analysis showed that the lignin content of corncob and bagasse material can not be eliminated completely through the process of delignification or pretreatment. Judoamidjojo et al. (1989) stated that the lignin is resistant to hydrolysis because of the aryl-alkyl and ester bond. The presence of difficult to degrade lignin content led to the formation of glucose becomes more difficult.

Table 1. Fiber composition analysis of corncob and sugarcane bagasse

Raw materials	Fibers	Content (%)		
		Before delignification	After delignification	Hydrolysis residue
Corncob	Cellulose	29,85	36,61	4,65
	Hemicellulose	32,67	11,17	1,33
	Lignin	12,74	2,99	2,71
Sugarcane bagasse	Cellulose	40,47	63,94	6,87
	Hemicellulose	17,35	7,67	0,47
	Lignin	22,87	1,50	1,33

The content of the initial cellulose of corncob material 29,85% lower than sugarcane bagassel materials of 40,47%. After the delignification process, there is an increase in the cellulose content of corncob and sugarcane bagasse materials respectively 36.61% and 63.94%. The increase in cellulose content is caused by the increase in the specific surface area of cellulose, and hemicellulose dissolution in NaOH. The increase in cellulose content of the material results of delignification followed by hemicellulose content decreased. There was a decrease in the hemicellulose content of corncob material from 32,67% to 11,17%. There was also a decrease in the hemicellulose content of the sugarcane bagasse material from 17,35% to 7,67%.

3.2 Sugars and ethanol production during the hydrolysis and fermentation process

Acid hydrolysis process is carried out using HCl and enzymatic hydrolysis using *Aspergillus niger* cultures with a density of 2×10^7 /ml and *Trichoderma viride* with a density of 3×10^7 /ml. Total sugar content analysis results indicate the kind of corncob, the addition of culture *A. niger* produce total sugar of 11,80%, higher than other treatments.

Table 2. Production of sugar and ethanol from substrate hydrolysis and fermentation of corncob and sugarcane bagasse

Raw materials	Treatments	Reducing sugar (%)	Total sugar initial (%)	Total sugar residue (%)	Substrate efficiency (%)	Ethanol content (%)
Corncob	Witout fungus	0,96 a	9,00 a	1,28 e	85,67 b	4,19 a
	<i>A. niger</i>	3,27 e	11,80 c	1,13 bc	90,35 cd	6,30 cd
	<i>T. viride</i>	2,49 c	10,60 bc	1,62 f	84,71 b	5,04 abc
	<i>A. niger</i> + <i>T. viride</i>	1,56 b	10,20 ab	1,72 g	83,13 a	4,43 ab
Sugarcane bagasse	Witout fungus	1,03 a	10,50 bc	1,11 ab	89,43 c	4,37 ab
	<i>A. niger</i>	2,92 d	11,20 bc	1,18 d	89,46 c	4,78 abc
	<i>T. viride</i>	3,99 f	16,90 e	1,07 a	93,67 e	6,83 d
	<i>A. niger</i> + <i>T. viride</i>	2,53 c	13,30 d	1,16 cd	91,26 d	6,09 bcd
LSD 5%		0,325	1,291	0,041	1,138	2,326

Values followed by the same letter showed that not significant different by Least Significant Difference Test (LSD) at 5% level.

For the type of sugarcane bagasse, the addition of culture *T. viride* produce total sugar of 16,90%, higher than the other treatments (Table 2). Average of all treatments using sugarcane bagasse materials produced higher total sugar than corncob, respectively 12,98% and 10,40%. It is influenced by the initial cellulose content of sugarcane bagasse is higher than with corncob, so the total sugar content is higher. A high content of total sugar will result in high reducing sugar. This is because more sugar polymers are available, it will be the more sugar that can be reduced to a sugar monomers.

Reducing sugar content analysis results indicate the type of corncob material, the addition of culture *A. niger* produces reducing sugar of 3,27%, higher than the other treatments (Table 2). Bioethanol research results conducted by Chen et al. (2007) that uses raw materials to the delivery of corncob cellulase and cellulase enzymes from *T. reesei* and *A. niger* on hydrolysis process is capable of producing up to 6.22% reducing sugar. Reducing sugar content which can be caused by less than optimal results delignification of cellulose content is low, amounting to 36,61%, while the results of the research by Sunarti et al. (2008) that cellulose content of corncob after delignification process by 44.36%. If the cellulose content as substrate hydrolysis is low, the reducing sugar produced less than optimal. Reducing sugar content analysis results indicate the type of sugarcane bagasse material, the addition of culture *T. viride* produces reducing sugar of 3,99%, higher than the other treatments (Table 2).

Substrate efficiency indicates the amount of sugar consumed during the fermentation process (Arnata et al., 2010). The higher value of substrate efficiency will decrease the residual sugar content. For the the type of corncob material, the addition of culture *A. niger* has a value of substrate efficiency of 90,35%, higher than the other treatments, while the addition of culture *A. niger* + *T. viride* has the lowest value of the substrate efficiency of 83.13% (Table 2). For the indicate the type of sugarcane bagasse material, the addition of culture *T. viride* has a value of substrate efficiency of 93,67%, higher than the other treatments, while the treatments without fungus addition has the lowest substrate efficiency value of 89,43% (Table 2). Average of all treatments using sugarcane bagasse materials produce substrate efficiency value is higher than the corncob materials, respectively 90,95% and 85,96%.

3.3 Fermentation activity

Ethanol production through fermentation using yeast *Saccharomyces cerevisiae* in anaerobic condition for eight days. In anaerobic condition or no oxygen, yeast will make the process of anaerobic fermentation where sugar is available to be converted into ethanol and CO₂. Khanal (2008) suggested that the anaerobic fermentation, organic substances catabolized without available oxygen which means there is no external electron acceptors but through the balance of internal oxidation-reduction reaction. Products generated during the process that electrons are released when organic matter breakdown. In the fermentation process, the substrate is only partially oxidized and produced only a little energy. At the time converted to pyruvic acid, glucose as a substrate to release electrons, but the electrons will then be taken by the pyruvate to ethanol.

Based on Figure 1 and Figure 2 shows the change in total sugars, ethanol content and pH value during fermentation in all treatments. During the fermentation process, total sugars decreased from 0 to 2 days, after which total sugars decreased relatively constant until 8 days. Decrease in total sugar content followed by an increase of ethanol content, it is due to the consumption of glucose by yeast to produce ethanol. Ethanol content increased on 2 days until 6 days, after which the ethanol content is relatively constant until the end of fermentation.

Judoamidjojo (1989) stated that the ethanol fermentation in anaerobic conditions using yeast, from one molecule of glucose to form two molecules of ethanol and CO₂. So theoretically one gram of glucose will produce 0,51 grams of ethanol. The pH value of substrate decreased from the beginning to the end of the fermentation process. The pH decline associated with the production of organic acid during fermentation (Rehm and Reed, 1983).

The total sugars content during the fermentation process as a whole has decreased. Sugar as fermentation substrate in anaerobic condition used by *S. cerevisiae* to produce ethanol. Therefore, the total sugar content of the substrate decreases with increasing fermentation time. Glucose as substrate fermentation is broken down into pyruvate through glycolysis and produces two molecules of ATP (Tao et al., 2005).

Total sugars significantly decreased occurred on 0 to 2 days. This suggests that the yeast *S. cerevisiae* actively utilize glucose as a substrate fermentation to produce ethanol. After 2 days, the rate of decline in total sugar relatively slowly. The research result by Didu (2010) showed that the decrease in sugar concentration occurred more rapidly in the initial phase to enter the 18 hours, and then after the 18 hours the rate is relatively slow decline in sugar concentration. It can be caused by the accumulation of ethanol as a product of fermentation. Clark and Mackie (1984) stated that 1-2% ethanol at a concentration sufficient to inhibit the

growth and the concentration of 10% may stop the growth of yeast. This is because ethanol is toxic to yeast, so that in most yeast, ethanol production will be halted at a concentration of 11-18%.

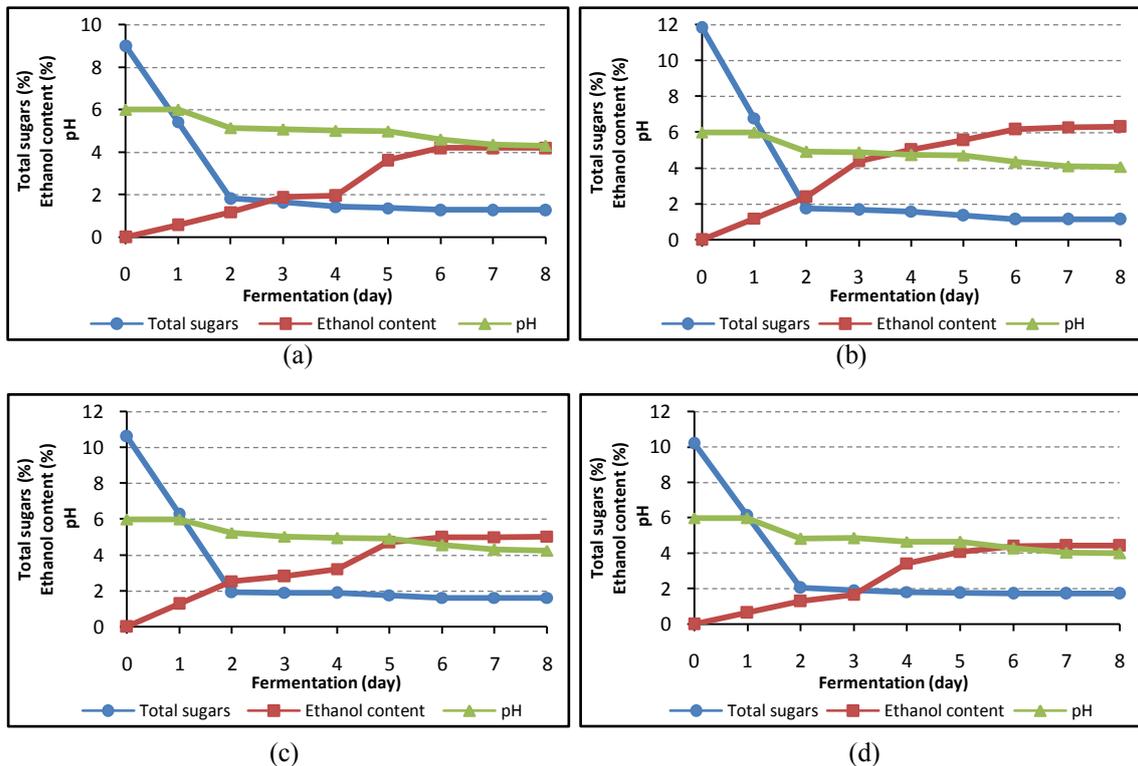


Figure 1. The changes in total sugars, ethanol content and pH value during fermentation on corn cob materials with: (a) without the fungus addition, (b) the addition of *A. niger*, (c) the addition of *T. viride*, (d) the addition of *A. niger* and *T. viride*.

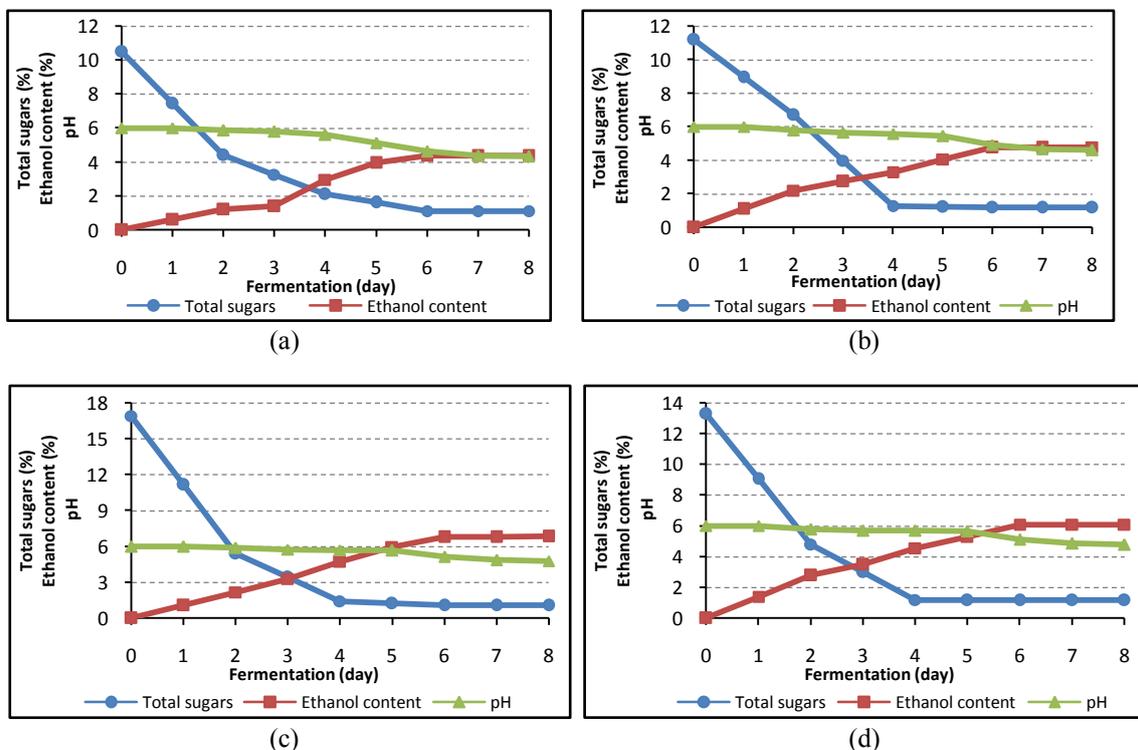


Figure 2. The changes in total sugars, ethanol content and pH value during fermentation on sugarcane bagasse materials with: (a) without the fungus addition, (b) the addition of *A. niger*, (c) the addition of *T. viride*, (d) the addition of *A. niger* and *T. viride*.

The sugar content in the fermentation substrate remaining until the end of the fermentation process. This show is not all sugar in the substrate utilized by the yeast *S. cerevisiae* to produce ethanol. Wignyanto and Novita (2001) stated that most of the sugar is not converted due to the sugar concentration outside the cell is too high, causing differences in the concentration and osmotic pressure of the fluid between the environment and the resulting yeast cells plasmolysis events.

During the fermentation process, the ethanol content increased in all treatments until the end of fermentation. This suggests that the anaerobic conditions in the form of glucose fermentation substrate utilized by *S. cerevisiae* to form products such as ethanol. Prasad et al. (2006) stated that the anaerobic fermentation, glucose is converted to pyruvic acid through the EMP pathway. Pyruvic acid is converted to acetaldehyde by the enzyme pyruvate decarboxylase assistance and subsequently converted into alcohol with the help of the enzyme dehydrogenase.

For the type of corn cob materials, the addition of culture *A. niger* has ethanol content of 6,30%, higher than the other treatments, while treatment without fungus addition has lower ethanol content of 4,19% (Table 2). For the type of sugarcane bagasse materials, the addition of culture *T. viride* has ethanol content of 6,83%, higher than the other treatments, while treatment without fungus addition has lower ethanol content of 4,37% (Table 2). Average of all treatments using sugarcane bagasse materials produce higher ethanol content than corn cob materials, respectively 5,52% and 4,99%.

3.4 Bioethanol production

Ethanol is derived from distillation process of fermentation substrates that have been separated by water. The volume of ethanol produced in the different treatment of each type of raw material and fungus culture For the type of corn cob materials, the addition of culture *A. niger* produce ethanol at 282,3 ml/kg, higher than the other treatments, while treatment without fungus addition has lower ethanol yield at 185,1 ml/kg (Table 2). For the type of sugarcane bagasse materials, the addition of culture *T. viride* produce ethanol at 299,2 ml/kg, higher than the other treatments while treatment without fungus addition has lower ethanol yield at 188,6 ml/kg (Table 2). Average of all treatments using sugarcane bagasse materials produce higher ethanol yield than corn cob materials, respectively 258,2 ml/kg and 237,3 ml/kg. Potential ethanol yield from raw material bagasse and corn cob respectively of 279 ml/kg and 358 ml/kg (Kuhad and Singh, 1993). This suggests that ethanol yield that have been obtained can still be improved.

Table 3. Distilled ethanol production

Raw materials	Treatments	Ethanol yield (ml/kg)	Spesific gravity
Corn cob	Witout fungus	185,1 a	0,830
	<i>A. niger</i>	282,3 cd	0,826
	<i>T. viride</i>	259,0 bcd	0,830
	<i>A. niger</i> + <i>T. viride</i>	222,8 abc	0,829
Sugarcane bagasse	Witout fungus	188,6 a	0,829
	<i>A. niger</i>	253,3 bc	0,826
	<i>T. viride</i>	299,2 d	0,822
	<i>A. niger</i> + <i>T. viride</i>	291,7 cd	0,825
LSD 5%		43,154	ns

Values followed by the same letter showed that not significant different by Least Significant Difference Test (LSD) at 5% level, ns = not significant.

Based on Figure 3 shows that in the treatment without the addition of fungus, the ethanol yield from corn cos and sugarcane bagasse is lower than the other treatments. In the addition of culture *A. niger*, the ethanol yield from corn cob material is higher than sugarcane bagasse. In the addition of culture *T. viride*, ethanol yield from sugarcane bagasse material is higher than corn cos. While on treatment the addition of culture *A. niger* + *T. viride*, ethanol yield from sugarcane bagasse material is higher than corn cob.

Effectiveness of fungus in increasing the ethanol yield from lignocellulosic materials depends on the activity of enzymes produced. *A. niger* produces more hemicellulase enzyme complex that can hydrolyze hemicellulose into glucose (Pham et al., 2010). While the advantages of *T. viride* compared to other types of fungus are capable of producing a complete cellulolytic enzymes and also produce enzymes xyloglukanolitik (Tribak et al., 2002).

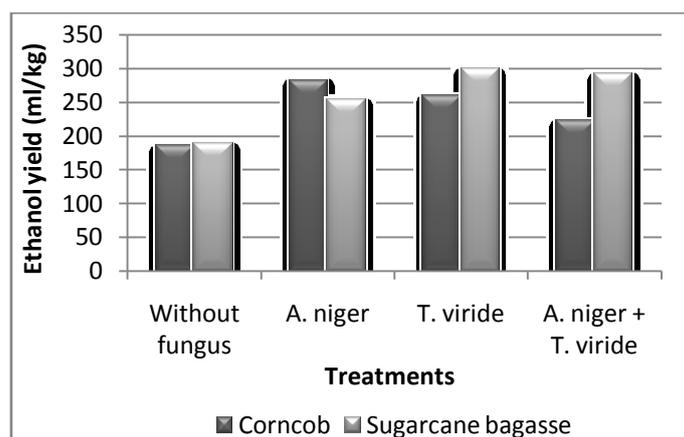


Figure 3. Volumes of ethanol at various treatments.

The pH value measurement results on all treatments showed a decrease. It is related to the consumption of glucose through the process of glycolysis and accumulation of organic acids compounds formed during fermentation, including acetic acid, lactic acid and pyruvic acid. Rehm and Reed (1983) states that the acid formed as a by-product of ethanol fermentation processes such as acetic acid, pyruvic acid and other organic acids play a major role in the decrease in pH. Low pH values at the end of fermentation showed that the accumulation of acid and accumulate more and more, due to the *S.cerevisiae* cells that can not convert the acids are mainly pyruvic acid into ethanol. Medium conditions such as pH has a great influence on the growth and activity of microorganisms.

Table 4. The average of pH value of the end of fermentation substrate on various treatments.

Treatments	pH value		
	Corncob	Sugarcane bagasse	Average
Without fungus addition	4,293	4,318	4,305
A. niger	4,074	4,613	4,343
T. viride	4,256	4,778	4,517
A. niger + T. viride	4,017	4,778	4,397

Based on Table 4 shows that only by using acid hydrolysis (without the fungus addition) will result in a lower pH value than the cellulolytic hydrolysis using a fungus culture. Chandel et al. (2007) stated that the use of acid saccharification is not specific. In addition to glucose, the acid saccharification can produce byproducts such as furan compounds, phenolic, and acetic acid. Acidic byproducts that if not removed can inhibit the fermentation process by lowering the pH value is lower.

IV. Conclusion

The addition of culture *Aspergillus niger* and *Trichoderma viride* on the hydrolysis of corncob and sugarcane bagasse effect on increasing of sugar production, ethanol yield and substrate efficiency. In the treatment of the raw material of corncob with variations the addition of fungus culture, the addition of *Aspergillus niger* is the best treatment combination that produces a total sugar content of 11,80% and the ethanol yield of 282,3 ml/kg with substrate efficiency of 90,35%. While the sugarcane bagasse materials, the addition of *Trichoderma viride* is the best treatment combination that produces a total sugar content of 16,90% and the ethanol yield of 299,2 ml/kg with substrate efficiency of 93,67%. Production of bioethanol from sugarcane bagasse materials at an average of all the fungus additional treatments produce higher yields than corn cobs with an average ethanol yield of 258,2 ml/kg.

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