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The Effect of pH on Simultaneous Saccharification and Fermentation Process of Water Hyacinth (*Eichhornia crassipes* (Mart.) Solms.) Using *Trichoderma harzianum* and *Saccharomyces cerevisiae*

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Abstract:- Research has been done on simultaneous saccharification and fermentation of water hyacinth (*Eichhornia crassipes* (Mart.) Solms.) with different pH to produce bioethanol. Simultaneous saccharification and fermentation process was the integration between saccharification or hydrolysis of cellulose into sugar and fermentation of sugar into ethanol, with utilized microorganism of *Trichoderma harzianum* and *Saccharomyces cerevisiae*. The results indicated that water hyacinth biomass could be used to produce bioethanol through simultaneous saccharification and fermentation (SSF) process. The highest concentration of bioethanol 47.20 $g.L^{-1}$ achieved by pH 5.0 treatment.

Keywords:- bioethanol, pH, simultaneous saccharification and fermentation (SSF), water hyacinth (*Eichhornia crassipes* (Mart.) Solms.)

I. INTRODUCTION

Excessive consumption and burning of fossil fuels emit great quantities of gases in the atmosphere, especially CO_2 (anthropic emissions). The rising emissions of this gas and others as methane (CH_4), nitrous oxide (N_2O), hydrofluorinecarbonates (HFCs), perfluorcarbonates (PFCs) and sulphur hexafluoride (SF6) in the atmosphere have caused serious environmental problems, accentuating the global warming [1].

During the last decade, the production of fuels from biomass materials as bioethanol received more attention in the worldwide [2]. The most bioethanol is produced by first generation processes based on fermentation technologies for sugar and starchy crops. However, these crops have some drawbacks: a high value for food application and low sugar yield per hectare. Thus, currently, bioethanol can be produced from inexpensive and abundant lignocellulosic biomass [3]. Suitable processes for lignocellulosic biomass are being developed under the name "2nd generation bioethanol processes" [4].

Bioethanol from renewable carbon sources (such as lignocellulosic biomass) are particularly attractive based on bioresource sustainability, ecofriendly, inexpensive and these resources do not compete directly with food production, or with land that may be needed for food production [5]. In principle, all lignocellulosics can be converted into simple sugars which can serve as useful raw materials in the production of bioethanol [6].

Production of bioethanol from lignocellulosic wastes has received widespread interest due to their availability, abundance and relatively low cost. Moreover, bioethanol is a clean-burning fuel that makes no net contribution to global warming [3]. Bioethanol represents closed carbon dioxide cycle because after burning of bioethanol, the released carbon dioxide is recycled back into plant material. Plants use carbon dioxide to synthesize cellulose during photosynthesis cycle. Bioethanol production process only uses energy from renewable energy sources, no net carbon dioxide is added to the atmosphere, making bioethanol an environmentally beneficial energy source. In addition, the toxicity of the exhaust emissions from bioethanol is lower than that of petroleum sources [7]. Water hyacinth (*Eichhornia crassipes* (Mart.) Solms.) can be utilized for this process of conversion to bioethanol. Water hyacinth is a monocotyledoneous freshwater aquatic plant, belonging to the family Pontederiaceae. It is considered as a noxious weed in many parts of the world as it grows very fast and depletes nutrient and oxygen rapidly from water bodies, adversely affecting flora and fauna. Moreover due to high evapotranspiration it adds to water crisis all over the places where it grows. Composition of water hyacinth estimated was found to be hemicellulose 42% , cellulose 30% and lignin 11% [15]. The possibility of converting water hyacinth fuel bioethanol is currently established in a number of developing countries [8], and can be utilised in Indonesia. The biological conversion of bioethanol from lignocellulosic biomass can be achieved by simultaneous saccharification and fermentation (SSF) process. This process (SSF)

plays an effective role to overcome enzyme inhibition. SSF combines hydrolysis with fermentation to keep the concentration of glucose low. The accumulation of bioethanol does not inhibit cellulose as much as high concentrations of glucose, so SSF is a good strategy for increasing the overall rate of cellulose to bioethanol conversion. In SSF process both cellulose hydrolysis and fermentation of glucose are carried out in presence of fermentative microorganisms in a single step and the process optimally operates at 37 to 38°C. This technique reduces the number of steps in the process, and is a promising way for converting lignocellulose to biethanol [9].

For microbial hydrolysis, *Trichoderma harzianum* can produce cellulolytic enzyme such as cellulase and hemicellulase. Three major groups of enzymes are involved in the hydrolysis of cellulose, namely, endoglucanase, exoglucanase and cellobiase, respectively. The endoglucanases attack randomly and cleave the cellulose chains to form glucose, cellobiose and cellotriose. The exoglucanases attack the nonreducing end of cellulose to form the cellobiose units. Finally, cellobiase converts cellobiose into D-glucose [9]. Meanwhile *Saccharomyces cerevisiae* is well known yeast for its fermentation capacity and hence can be employed for alcohol production from various sugar containing materials [10].

According [11] the degree of acidity (pH) is one of several important factors that can affect the bioethanol fermentation process. His study states that the highest production of bioethanol from bagasse conversion to bioethanol using enzyme of xylanase through the SSF is the condition of the degree of acidity (pH) 5 to produce bioethanol concentration of 27.1 $g.l^{-1}$. [12] in his research stated that fungi degrade cellulose highest at pH 5.0 is 85.9 $g.L^{-1}$. According [13] the manufacture of bioethanol from elephant grass (*Pennisetum purpureum*) by hydrolysis enzyme from *Clostridium thermoacticum* bacteria produce the highest bioethanol (24.5 – 56.9 $g.L^{-1}$) at pH 7. The highest levels of bioethanol (68.5 $g.L^{-1}$) produced by cassava using the enzyme of α-amylase, β-glucosidase and the yeast of *S.cerevisiae* obtained at pH 5.5 [14].

Based on the content of cellulose in water hyacinth is large enough, the water hyacinth can be used as raw material for the production of bioethanol. Making bioethanol from water hyacinth can be done through simultaneous saccharification and fermentation with utilize *T. harzianum* into account as a source of cellulolytic enzymes and *S. cerevisiae* as an appliance for alcohol fermentation from saccharified liquor extracted.

II. MATERIALS AND METHODS

A. The Raw Material

The study was conducted from May to June 2012**.** The water hyacinth was collected from local ponds around the campus of State Islamic University of Sunan Gunung Djati, Bandung, Indonesia, pure culture of *T. harzianum* and *S. cerevisiae* were obtained from the microbiology laboratory of Institute of Technology Bandung, PDA (Potato Dextrose Agar), distilled water, alcohol, buffered phosphate pH 5.5, PDB (Potato Dextrose Broth), HCl, NaOH, 1% NaOCl, NaOH 15%, NPK, ZA.

B. The Experimental Design

The design of experiments in this study was using completely randomized design with variations in pH as treatment consisting of 9 levels were: incubation at pH 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0 respectively. Replication was done 3 times, so the total number of experimental units were 27 units.

C. Preparation of Water Hyacinth

The water hyacinth was thoroughly washed several times with tap water to remove adhering dirt, chopped into small pieces of size 1-2 cm (approx), and further grounded to even smaller particles of size 1-2 mm (approx), and finally dried in a hot air oven at 106 °C for 6 hours. The dried material was stored at room temperature until used. Delignification of materials were using 1% NaOCl for 5 hours at 28 ºC. Material was washed several time with aquadest and then soaked in 15% NaOH for 24 hours at 28 ºC. Then material was dried at 50 ºC for 48 hours, finally obtained substrate of water hyacinth.

D. Simultaneous Saccharification and Fermentation (SSF)

A steam-autoclaved (121^{o}C, 1 atm for 15 min) suspension of water hyacinth substrat (12.5 g) in 40 ml phosphate buffer and 40 ml nutrient of Potato Dextrose Broth, added with NPK and ZA fertilizers 0.04 g and 0.15 g respectively, pH 5.5 was used as SSF medium. SSF experiments were started by inoculation with 10 % (v/v) of *T. harzianum* and *S. cerevisiae* respectively under room temperatur for 3 days. The enzymatic hydrolysis was undertaken at the same time as the fermentations that were carried out in 125 mL Erlenmeyer flasks.

E. Analytical method

The amount of released glucose and cellubiose were measured with glucose analyzer from Yellow Springs Instruments [16]. Ethanol produced in the fermentation medium was estimated by potassium dichromate oxidation method. Acid dichromate solution (0.1 M $Cr_2O_7^{2-}$ in 5 M H_2SO_4) was prepared by

dissolving 7.5g of potassium dichromate in dilute sulfuric acid and the final volume was adjusted to 250 mL with deionized water. To prepare the calibration curve, 300 µL of ethanol solutions were filled into small plastic caps and placed into beakers containing 3 mL of acid dichromate. The beakers were tightly sealed with parafilm and kept at room temperature for 30 min. The maximum absorbance was recorded at 590 nm [17]. The mean value of all datas were compared by variances analysis (ANOVA) and then Duncan's Multiple Range Test (DMRT) for pair wise comparison was used at the 5% significance level [18].

III. RESULTS AND DISCUSSIONS

The production of ethanol from non-starch, lignocellulosic materials such as water hyacinth biomass is, however, a fairly recent development. The cellulose conversion option that many currently favor is SSF process. In this process, the cellulose hydrolysis and glucose fermentation steps are combined in a single vessel. Since cellulase is inhibited by glucose as it is formed, rapid conversion of the glucose into ethanol by yeast results in faster rates, higher yields, and greater ethanol concentrations than possible for other process. The SSF of cellulose to ethanol combines the action of two microorganism (*T harzianum and S cerevisiae*).

The table 1 showed that pH treatment which produces the highest concentration of bioethanol was pH 5.0 treatment with an average ethanol concentration of 47.20 $g.L^{-1}$. This indicated that the pH 5.0 treatment was optimum pH at the SSF. Meanwhile, pH treatment that produced the lowest concentration of bioethanol was pH 3.0 treatment with an average ethanol concentration of 13.17 $g.L^{-1}$. This was occured because the pH treatment created too acidic conditions. In this conditions, *T. harzianum* and *S. cerevisiae* could not optimally work so that the content of bioethanol has the lower levels when compared with other pH treatments.

The degree of acidity (pH) was one of several important factors that could affect the fermentation process of ethanol [11]. In this study, pH 5.0 treatment produced the higher ethanol content compared with other pH treatments. This was occured because, *T. harzianum* and *S. cerevisiae* can optimally work. According [2] yeasts like *S. cerevisiae* can generate in pH 4-5, while mold like *T. harzianum* can generate in the optimum pH 5-7. In the SSF, the pH conditions greatly affected the production of bioethanol content because the process involved two microorganisms, namely mold and yeasts that have different pH ranges to survive in the medium. This reinforces that the SSF process using *T.harzianum* and *S. cerevisiae* could still produce bioethanol, although the levels produced were not too large. This showed that in the pH range of *T. harzianum* and *S. cerevisiae* was able to grow and still be able to work to produce the final product.

Table 1. Residual concentration of sugars and maximum ethanol production in SSF process.

pH	Concentration ($g.L^{-1}$)		
	Cellobiose	Glucose	Ethanol
3.0	0.19	0.64	13.17(a)
3.5	0.15	0.53	20.20(b)
4.0	0.10	0.61	29.17(c)
4.5	0.00	0.54	39.40(de)
5.0	0.00	0.52	47.20(f)
5.5	0.08	0.59	41.80(e)
6.0	0.08	0.64	36.80(d)
6.5	0.10	0.61	29.67(c)
7.0	0.12	0.64	23.60(b)

Description : (numbers followed by different letters indicate difference at 5% level test).

The SSF process was preceded by 3 days-enzymatic microorganism, after which the concentrations of glucose and cellobiose generated from different pH treatments were determined (Table 1). While pH 4.5 and pH 5.0 treatments produce exclusively glucose, pH 3.0, 3.5, 4.0, 5.5, 6.0, 6.5 and 7.0 treatments generate both glucose and cellobiose. It is likely that the amount of glucose produced in pH 3.0, 3.5, 4.0, 5.5, 6.0, 6.5 and 7.0 treatments led to the inhibition of β-glucosidase, leaving a residual cellobiose concentration. According to [19], β-glucosidases are strongly inhibited by glucose, while endo- and exoglucanase are inhibited by cellobiose. The difference between the produced sugar concentrations with pH 3.0, 3.5, 4.0, 5.5, 6.0, 6.5 and 7.0 treatments in comparison with pH 4.5 and pH 5.0 treatments were due to the type of cellulose and the enzymatic activities present in *T.harzianum* and *S. cerevisiae*, According [4] mold could still survive at pH 3 to 8.5, while yeast in the range of 2.5 to 8.5.

The ethanol production was monitored for 3 days of SSF process (Table 1). As it can be expected pH 5.0 was the best treatment for SSF process, resulting in higher values of ethanol concentration when compared with the other pH treatments, since the pH 5.0 treatment resulted in lower available fermentable sugars than the other pH treatments. Cellobiose, glucose and ethanol concentrations at the end of the SSF process, for the pH

treatments were investigated water hyacinth. In pH 4.5 and pH 5.0 treatment, celobiose was totally converted in glucose at the 3th day after cell inoculation. The presence of glucose in the medium at the end of the SSF process indicates the continuation of the catalytic activity of the celullase complex [20], as it was noticed in the experiments herein done or increased glucose concentrations were due to the inability of yeast to ferment glucose at lower pH.

IV. CONCLUSIONS

The substrate from water hyacinth could be used as ethanol production through simultaneous saccharification and fermentation process. Based on the study showed that the variation of pH provide significant effect on bioethanol concentration. The pH 5.0 treatment was the optimum pH for maximum ethanol content (47.20 $g.L^{-1}$). Therefore, simultaneous saccharification and fermentation process of water hyacinth biomass for ethanol production was carried out in a high yield by optimum pH treatment and appropriated mold and yeasts. Bioethanol from water hyacinth produced in restored or constructed wetlands may also be recognized as a replicable example of mitigation and adaptation strategies to cope with the progression of regional and global environmental changes and the high cost or depletion of petroleum in the near future.

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