HYDROGEN PRODUCTION FROM RICE STRAW BY NONPHOTOSYNTHETIC ANAEROBIC FERMENTATION THROUGH ENZYMATIC HYDROLYSIS

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ABSTRACT

The ever increasing need of the worldwide energy and the decreasing of reserves of fossil fuels lead to the increase of price of fossil fuel which suffers almost all sectors of human life. Furthermore, a negative effect to the environment as a result of combustion of fossil fuels that emits CO_2 threats the future of world life. For these reasons, it is necessary to find new sustainable and environmental friendly energy sources that could substitute fossil fuel. Hydrogen becomes one of attractive attention because its combustion releases a high energy of about 120.1 MJ/kg and produces water vapor only. Biohydrogen has been produced by using variety of simple carbohydrates, however these kind of raw materials are expensive not economically feasible. At the same time, Indonesia produces 180 million ton of rice straw per year as solid waste. Rice straw contains 47% cellulose and 35% hemicellulose that can be hydrolyzed to glucose and xylose, respectively, and then can be fermented to biohydrogen.

The objective of this work is to produce hydrogen from rice straw. Production of cellulase enzyme that can effectively hydrolyze the rice straw, various operating condition on the enzymatic hydrolysis of rice straw and fermentation of rice straw hydrolysate were investigated. This work also investigated the kinetic and hydrodynamic of hydrogen fermentation in stirred tank reactor.

Cellulase enzyme was prepared in solid fermentation by single and combined culture of *Trichoderma reesei* and *Aspergillus niger* under various ratio. Before hydrolyzed, rice straw was delignified by 2% NaOH at 80 ^{o}C for six hours. Hydrolysis was conducted in a beaker glass equipped with heater and stirrer. The kinetic of hydrogen fermentation from hydrolysate was investegated in four parallel of 500 mL batch reactor under various temperatures and initial substrates. The mixture of glucose and xylose with a ratio of 1.5 : 1 was used as hydrolysate model. The effect of stirring speed on hydrogen fermentation was investigated in 5 L stirred tank reactor by using *Enterobacter aerogenes* NBRC 13534 with glucose as substrate. Simulation of hydrodynamic factor was conducted using Computational Fluid Dynamic ANSYS FLUENT 13.0.

From study of hydrolysis, it was found that: (1) Rice straw was a better substrate for cellulase production than corn stem by which cellulase activity produced by this substrate was 23– 47% higher. (2) The highest enzyme activity produced by *T. reesei* and *A. niger* were 1.66 U/mL and 1.69 U/mL, respectively attained during 6 day and 8 day incubation, using rice straw as the substrate. (3) The enzymes produced by *T. reesei* and *A. niger* consisted of several types of enzyme (crude enzyme). These enzymes are needed to produce reducing sugar. Enzyme samples from *T. reesei* have cellulase and xylanase activity of 1.29 U/mL and 4.45 U/mL, respectively whereas enzyme from *A. niger* have cellulase activity of 0.73 U/mL and xylanases activity of 2.37 U/mL. The enzymes have the highest activity at pH 3.0 and temperature of 50 – 60 °C. However, the enzymes most stable at pH 5.5 and temperature 40 °C. (5) The mixture of enzymes showed better performance compared to the enzyme from *T. reesei* and *A. niger* including pure enzyme especially at the ratio T/A = 2/1. Increasing the enzyme concentration from 0.47 to 0.93 g/L increased reducing sugar concentration from 6.4 to 8.7 g/L for 7 hours hydrolysis.

From study of fermetation, it can be concluded that: (1) the hydrogen fermentation from a mixture of glucose and xylose is affected by the initial substrate concentration and temperature and was optimum at initial substrate concentration of 16.7 g/L and 30 ^oC under which the highest yield of 0.356 mol H_2/mol reducing sugar was obtained. (2) Hydrogen fermentation from glucose and xylose mixture using *E. aerogenes* showed the occurance of the the substrate inhibition. (3) The performance of hydrogen fermentation from natural hydrolysate was similar to that using synthetic hydrolysate. (4) The Andrew model was found to be suitable for hydrogen fermentation from glucose and xylose mixture. At temperature of 30 °C it was found that:

Equation of cell growth: $\mu = \frac{4\, S_o}{37{,}5 + S_o + \frac{S_o^2}{10}}$ hour^{-1}

Equation of H_2 production: $r_p = \frac{1{,}67\, S_o}{1{,}7 + S_o + \frac{S_o^2}{5{,}2}}$ mmol H_2/L.h

(5) Hydrogen fermentation is affected by the speed of stirring. Increasing the stirring speed from 46 to 165 rpm increased the hydrogen yield from 0.025 to 0.085 mol H_2/mol glucose.

Simulation showed that (1) the highest turbulence intensity occured at the impeller region, by which by increasing the stirring speed, the turbulence intensity in all part of the reactor will be more uniformly distributed. This will result in the diffusion of substrate to the cell surface and increasing the rate of biogas flowing out from liquid bulk. (2) Simulation also showed the volume fraction of biogas in the botton of the tank decrease by increasing the stirring speed. This shows that more gas go to surface and leave the reactor.