EFFECT OF TEMPERATURE AND pH ON SWEET POTATO STARCH RESIDUE FERMENTATION TO PRODUCE BIOHYDROGEN THROUGH DARK-FERMENTATION USING YEAST

Fajar A. Pamungkas [*] ***and Rudiana Agustini***
Department of Chemistry, Faculty of Mathematics and Natural Science
University State of Surabaya, Jl. Ketintang, Surabaya, 60231
e-mail: roxas_he@yahoo.co.id

Abstrak. *Telah dilakukan penelitian yang bertujuan untuk mengetahui pengaruh variasi temperatur fermentasi dan pengaruh pH fermentasi terhadap produksi biohidrogen melalui* dark-fermentation *dengan substrat limbah padat tapioka menggunakan kultur ragi. Metode yang digunakan dalam penelitian ini adalah metode fermentasi dalam sistem* batch *dimana biohidrogen hasil fermentasi diukur melalui metode analisis kromatografi gas. Variabel yang diujikan adalah temperatur ruang, 30 ^{o}C, 35 ^{o}C, 40 ^{o}C, dan 45 ^{o}C untuk pengaruh temperatur dan pH 4,0; 5,0; 6,0; dan 7,0 untuk pengaruh pH. Hasil uji statistik anova satu jalur menunjukkan adanya perbedaan rata-rata yang signifikan pada perlakuan temperatur ($p = 0{,}000 < 0{,}05$) dan adanya perbedaan rata-rata yang signifikan pada perlakuan pH ($p = 0{,}022 < 0{,}05$). Dapat diartikan bahwa temperatur dan pH mempengaruhi produksi biohidrogen melalui fermentasi gelap. Temperatur optimum untuk memproduksi gas biohidrogen adalah 27 ^{o}C dengan perolehan sebesar 16,4230% dan pH optimum 5,0 dengan perolehan 7,1458%.*
Kata kunci: dark-fermentation, *hydrogen, limbah padat tapioka, ragi*

Abstract. *Effect of fermentation temperature and fermentation pH on sweet potato residue fermentation using yeast by dark fermentation to produce biohydrogen was conducted. This experiment used fermentation method in batch system and the produced hydrogen measured by gas chromatography analysis method. The variable tested was room temperature, 30 ^{o}C, 35 ^{o}C, 40 ^{o}C, and 45 ^{o}C to investigate the effect of temperature and pH 4,0; 5,0; 6,0; dan 7,0 to investigate the effect of fermentation pH. Statistical test result showed that there was a significant difference in temperature variations ($p = 0{,}000 < 0{,}05$) and there was a significant difference in pH variations ($p = 0{,}022 < 0{,}05$). Temperature and pH affecting biohydrogen production through dark fermentation. The optimum temperature to product biohydrogen was 27 ^{o}C (16,4230% biohydrogen yield) and optimum pH at 5,0 (7,1458% biohydrogen yield).*
Keywords*: dark-fermentation, hydrogen, potato starch residue, yeast*

INTRODUCTION

The environmental pollution due to the use of fossil fuels makes it necessary to find alternative energy sources that are environmentally friendly and renewable. There are many of alternative energy being developing to substitute and decrease the uses of fossil fuels. Hydrogen fit the most of all because it produces only water when it is combusted or converted to electricity [1].

There are many ways to produce hydrogen. Most of them using dangerous chemicals, takes a lot of time, and inefficient to do due to its rarity of the materials. The easiest way to produce hydrogen is by fermentation.

Fermentative-method hydrogen production require common materials and easy to do. It is also efficient to be laboratory-scale conducted. There are several fermentative-method hydrogen production known, which is direct photolysis, photofermentation, indirect photolysis, water-gas shift reaction, two-phase fermentation, and high-yield dark-fermentation [2].

Among the different ways of fermentation-method hydrogen production, dark-fermentation has the following advantages:

(1) Has the potential to convert biomass or biowastes into hydrogen;
(2) Has a high rate hydrogen production;
(3) Has feasibility of effective process design and control [3].

Many factors can affects biohydrogen production through dark-fermentation. There are substrates, concentration, pH, and temperatures. Temperature can affect the hydrogen producing bacteria activity by influencing the activity of some important enzymes, such as hydrogenases.

Fermentation pH also affected the production of hydrogen. In acidic state, the fermentation path might be changed from acidogenic phase to alcohol-forming fermentation. It caused the producing of hydrogen decrease.

Manipulation on dark-fermentation technology is needed to producing biohydrogen efficiently and decreasing the effect of fermentation wastes.

MATERIALS AND METHOD

1. Fermentor preparation

500 mL reagent bottles washed and dried until no water left inside the bottles. The bottles then closed with the matching bottle caps, sealed with tape, and drown into the water bucket to checked gas leaking.

2. Substrate preparation

Sweet potato starch residue was strained until it had a same 100 mesh size and heated inside the oven in temperature 150 ^{o}C for 30 minutes. Then weighted 35 grams for each bottles as a substrate and diluted into hot water until it reached 35% substrate.

3. Determination of Optimum hydrogen fermentation temperature

The prepared substrate inoculated with the mixture of yeast and baker's yeast. The mixed culture nitrogen source was given in form of urea. After the inoculation, the fermentor was sealed to avoid gas leaking. The fermentation incubated for 12 hours in room temperatures, 30 ^{o}C, 35 ^{o}C, 40 ^{o}C, and 45 ^{o}C in order to obtain the optimum fermentation temperature. The produced gas then analyzed using Gas Chromatography.

4. Determination of Optimum hydrogen fermentation pH determination

The prepared sunstrate inoculated with the mixture of yeast and baker's yeast. The mixed culture nitrogen source was given in form of urea. After the inoculation, the fermentor was sealed to avoid gas leaking. The fermentation incubated for 12 hours in the optimum temperature that had been determined in previous method with pH variation of 5,0; 6,0; 7,0; 8,0. Buffer solution is needed to conditioning the fermentation system. The produced gas then analyzed using Gas Chromatography.

RESULTS AND DISCUSSION

1. *Effects of temperature on hydrogen production*

Fermentative hydrogen production at five temperatures room temperature, 30 ^oC, 35 ^oC, 40 ^oC, 45 ^oC was chosen to find out how increased temperature makes effect on fermentative hydrogen production. The fermentation was incubated for 12 hours at different temperatures.

The result showed that the optimum temperature to conduct fermentative hydrogen production was at room temperature (Table 1). The amount of biohydrogen yield produced was 16,42% higher than 30 ^oC, 35 ^oC, 40 ^oC, and 45 ^oC with hydrogen yield 14,35%, 9,32%, 5,80%, and 4,43% respectively.

Statistical result showed that the data had a significant difference ($p = 0,000 < 0,05$). It means that temperature affected biohydrogen production through dark fermentation. The highest hydrogen yield was produced in 27 ^oC with amount of 16,42%. The amount of biohydrogen yield was decreased in temperature increasing.

Microorganism need more time to adapt to the high fermentation temperature [4]. It takes time to the microorganism to start fermenting the substrate into products which affecting the biohydrogen produced.

Organic acids as a byproduct was Also affecting the biohydrogen production. According to the theory, 4 mole H_2/mole glucose production rate was achieved when the byproduct is acetate and 2 mole H_2/mole glucose was achieved when the byproduct is butirate [2][5][6].

Acetate/butirate production decreases as the temperature increases. It makes the biohydrogen produced decreases as well. Acetate production started to decrease within 35 ^oC-55 ^oC and makes the hydrogen produced to decrease [7].

The increase of fermentation temperature was also affecting some essential hydrogen producing enzymes like hydrogenases. As the temperature goes up, the enzyme activity decreases and biohydrogen producing activity went down as well.

2. *Effects of pH on hydrogen production*

Fermentative hydrogen production at four conditions 5,0; 6,0; 7,0; and 8,0; was chosen to find

Table 1. Biohydrogen Produced in Fermentation Temperature Variations

Temperature (^oC)	Biohidrogen (%)			
	Replication 1	Replication 2	Replication 3	Mean
27	18,23	15,52	15,48	16,42
30	15,08	13,99	13,99	14,35
35	10,85	9,22	7,89	9,32
40	5,73	5,68	6,03	5,80
45	5,23	5,11	2,95	4,43

Table 2 Biohydrogen Produced in Fermentation pH Variations

pH	Biohidrogen (%)			
	Replication 1	Replication 2	Replication 3	Mean
5,0	9,46	6,906	5,0715	7,1458
6,0	10,08	4,82	6,2770	7,0590
7,0	4,60	5,69	0,9099	3,7333
8,0	1,50	0,44	0,7359	0,8920

out how pH affecting fermentative hydrogen production. The fermentation was incubated for 12 hours at room temperature.

The result showed that the optimum fermentation pH to produce biohydrogen was at 5,0 pH condition (Table 2). The amount of biohydrogen produced was 7,1458% higher than 6,0; 7,0; and 8,0 with biohydrogen yield of 7,0590%, 3,7333%, and 0,8920% respectively.

Statistical result showed that the data had a significance difference ($p = 0{,}022 < 0{,}05$). It means that pH affected biohydrogen production through dark fermentation. The highest biohydrogen yield was produced at pH 5,0 with amount of 7,1458%. The amount of biohydrogen yield was decreased in pH increasing.

Highest biohydrogen production achieved when the pH condition was in 5,0-6,0 [4]. It occurred because of the increasing of VFA and ethanol as the byproduct of fermentation. High fermentation pH triggered acetate and propionate production and inhibited butirate production causing organic acid accumulation.

The inhibition occured because of the organic acid accumulation. The accumulation of organic acid causing the fermentation pH to drop and inhibits microorganism's reproduction cycle [8].

CONCLUSIONS

According to statistical test, fermentation temperature affecting fermentative biohydrogen production by yeast culture. Fermentation pH also affecting fermentative biohydrogen production by yeast culture. The optimum fermentation temperature to operate fermentative biohydrogen production was at room temperature (27 ^{o}C). The increasing of temperature showed decreasing biohydrogen production trends. The optimum fermentation pHto operate fermentative biohydrogen production was at pH 5,0. The increasing of pH showed decreasing biohydrogen production trends.

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