

## Bio-hydrogen Production from Vinasse By Using Agent of Fermentation Photosynthetic Bacteria *Rhodobium marinum*

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### Abstract

The aim of this research was to find out the effect of substrate concentrations (COD) of vinasse and the length of fermentation time to bio-hydrogen gas production using agent of fermentation photosynthetic bacteria, *Rhodobium marinum*. The production of bio-hydrogen was examined by varying COD of vinasse (10,000; 20,000; 30,000; 40,000; 50,000 mg COD/L) at certain fermentation time in the third, sixth and ninth day. The highest Hydrogen gas was obtained at ninth day of fermentation (82.66±18.6 mL). The highest Hydrogen Production Rate (HPR) and COD removal rate were obtained at concentration 50,000 mg COD/L, namely 109.98 mL H<sub>2</sub>/L/d and 1437.66 mg COD/L/d, respectively. Thus it can be concluded, the concentration of substrates (COD) from vinasse and the length of fermentation time have an effect on production of bio-hydrogen gas using *Rhodobium marinum*.

### Keywords

Bio-hydrogen, Vinasse, *Rhodobium marinum*

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## 1. INTRODUCTION

Hydrogen has long been considered as a fuel and alternative energy future. Hydrogen is a clean fuel because it does not generate CO<sub>2</sub> gas emissions and can be easily used as a "fuel cell" to generate electricity. In addition, hydrogen has a high energy of 122 kJ/g, which is 2.75 times larger than hydrocarbon fuels. There are several conventional methods of hydrogen production including steam reforming of methane (SRM), with other hydrocarbons (SRH), a non-catalytic partial oxidation of fossil fuels (POX) and auto thermal reforming combining SRM and POX. Some of these methods require energy and the high temperatures (>850 °C) (Kapdan and Kargi, 2006). Combustion of hydrogen will not cause the greenhouse effect, ozone depletion, or acid rain. The process of combustion produces only vapour and heat energy (Nath and Das, 2004).

The producing of renewable energy economically should use cheap and readily available substrates so it can be used on an industrial scale. Organic waste can be used as a substrate which is economical to produce bio-hydrogen. For example, urban waste, agricultural waste, solid and liquid waste from the organic industry (Das and Veziroglu, 2008). The advantages of using waste as a substrate, it can reduce

the accumulation of wastes in the environment. The example of potential waste for the production of bio-hydrogen is vinasse. Vinasse is a liquid waste product of the distillation ethanol production through fermentation of molasses. dos Reis et al. (2015) mentions the COD content of vinasse is 42,818 mg/L. In general, vinasse has a low pH, blackish brown color contains many residues, organic and inorganic components. Phenolic components (such as humic acid and tannic acid), melanoidin (the result of a reaction between sugars and proteins by Maillard reaction), caramel and furfural components contributing gives the color of vinasse. Some of these components which cause vinasse complex and difficult to be degraded. The amount of vinasse are overflow in this world, the distillation process of 110,000-120,000 tons molasses can produce 70,000 vinasse tons per year (Vaccari et al., 2005). In 2008, the production of vinasse in the world reaching more than 650 billion L Arimi et al. (2015). The research on using vinasse for bio-hydrogen production has not been done, some research of bio-hydrogen production from vinasse have been carried out by Júnior et al. (2015) which produced bio-hydrogen from vinasse with *Clostridium* bacteria and *Pectinatus* using the pack bed reactor with a working volume of 2.3 L. The results

was quite high, 509.5 mL H<sub>2</sub>/d/L. Later studies of Buitrón et al. (2014) bio-hydrogen produced about 57.4 mL H<sub>2</sub>/L/h using tequila vinasse as a substrate with thermal pre-treat anaerobic sludge as an inoculum bio-hydrogen producer. Lazaro et al. (2014) produced hydrogen 1.72-2.23 mmol H<sub>2</sub>/g CODinfluent using vinasse with microbial consortia at mesophilic temperatures. The aim of this research is to find out the effect of variation substrate concentrations of vinasse to bio-hydrogen gas production by using photosynthetic bacteria *Rhodobium marinum*.

## 2. EXPERIMENTAL SECTION

### 2.1 Materials

The microorganisms used in this research was *Rhodobium marinum*. The stock culture was obtained from NBRC (NITE Biological Resource Center) with the collection number 100434. Substrates used in this research were vinasse obtained from PT. Madu Kismo, Yogyakarta, Indonesia.

### 2.2 Methods

#### 2.2.1 Characterization of Vinasse

Characterization vinasse was conducted by measuring the Chemical Oxygen Demand (COD) (58,433 mg/L) following SNI method (SNI, 2009), Total Nitrogen (83.10 mg/L) and Total Organic Acids (11.15%) were analyzed following AOAC method (of Official Analytical Chemists and of Official Agricultural Chemists, US) and pH (3.63) were examined using pH meter (Jenway 3505).

#### 2.2.2 Pre-treatment of Vinasse

Pre-treatment were done in various stages of treatment such as by filtration, adjusted pH at 8 using NaOH 10 N and sterilization by autoclaving (121 °C in 15 minutes).

#### 2.2.3 Bio-hydrogen Production

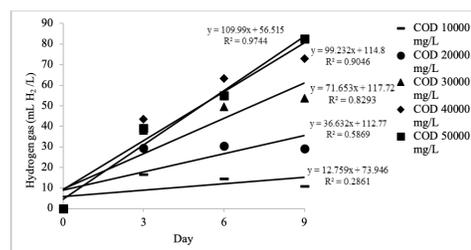
Bio-hydrogen production was conducted by using a variation of COD concentration of vinasse. In this research variation of COD used were 10,000; 20,000; 30,000; 40,000; 50,000. Photofermentation was performed using Scott bottles of 120 mL, with a working volume of substrate 80 mL. Substrate which has been pre-treated were inoculated with *R. marinum* (optical density ± 0.2). After inoculation, the substrate bottles were placed on a shaker at 120 rpm and irradiating fluorescent lamp (tubular lamp, Philips) 60 watt/m<sup>2</sup> at room temperature (30 °C). Fermentation was carried out for third, sixth and ninth days.

#### 2.2.4 Analytical Methods

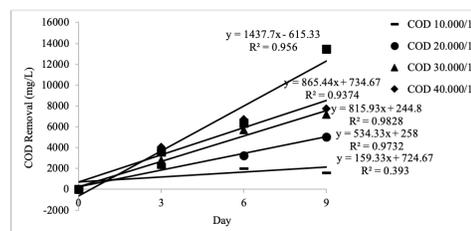
Hydrogen gas were determined by Gas Chromatography using a thermal conductivity detector (TDC) with pora-packed column. Oven temperature were set at 250 °C and 150 °C respectively. The flow rate of carrier gas was used as a standard. Calculations for composition of gases were accomplished using comparison area between samples and standard. COD assays were determined according to SNI method (SNI, 2009).

## 3. RESULTS AND DISCUSSION

The linier regression of Bio-hydrogen gas and COD removal at various concentration were presented in Figure 1 and 2. The result of hydrogen production rate and COD removal rate were presented in Table 1 dan 2, then the chemical properties of substrate after production was presented in Table 3.



**Figure 1.** Linier Regression of Bio-hydrogen gas at Various COD Concentration (10,000-50,000 mg/L)



**Figure 2.** Linier Regression of COD Removal at Various COD Concentration (10,000-50,000 mg/L)

### 3.1 Pre-treatment of Vinasse

Pre-treatment of vinasse was carried out so it can be an effective substrate for the production of bio-hydrogen by *R. marinum* bacteria. Pre-treatment was carried out in various steps and finished with filtration, adjusting pH to 8 using 10 N NaOH and sterilizing using autoclaves (121 °C for 15 minutes). Filtration was carried out so the granules or solids are discarded. Adjusting pH to 8 because the optimum pH for growth of *R. marinum* is 6.5 - 8.5. After adjusting the pH, sterilization was done using an autoclave (121 °C for 15 minutes). Sterilization was needed to destroy all microbes that were not needed, so only *R. marinum* bacteria which should be able to produce bio-hydrogen.

Pre-treatment must be done because vinasse has the characteristics of having many solids and very acidic. The color of vinasse before and after pre-treatment was not change, the color of vinasse was remains blackish brown. The research of dos Reis et al. (2015) states that the COD content of vinasse was 42,818 mg/L. In general, vinasse has a low pH, a blackish brown color that contains many residues, organic and inorganic components. Phenolic components (such as humic acid and tannic acid), melanoidin (the result

**Table 1.** Result of Bio-hydrogen Production of Various COD Concentration (10,000-50,000 mg/L)

COD Conc. (mg/L)	Days	Bio-hydrogen Production	
		Hydrogen gas (mL)	Hydrogen Production Rate (mLH <sub>2</sub> /L/d)
10,000	3	16.63 ± 3.08	12.76
	6	14.47 ± 1.55	
	9	10.92 ± 0.87	
20,000	3	29.49 ± 3.23	36.63
	6	30.31 ± 3.26	
	9	29.03 ± 6.57	
30,000	3	37.96 ± 2.93	71.65
	6	49.36 ± 6.84	
	9	53.52 ± 2.67	
40,000	3	43.52 ± 8.37	99.23
	6	63.32 ± 2.54	
	9	72.78 ± 13.2	
50,000	3	38.91 ± 0.68	109.98
	6	54.88 ± 16.4	
	9	82.66 ± 18.6	

of the reaction between sugar and protein by the maillard reaction), caramel and furfural components that contribute to give color to vinasse. Some of these components cause complex vinasse and are difficult to degrade. According to [García-Sánchez et al. \(2018\)](#) the COD content in vinasse tequila was  $22,085 \pm 1325$  mg/L. the organic components of vinasse tequila were carbohydrates (7.4 g/L), lactate (4.7 g/L) and other short chain organic acids (2.6 g/L). Then according to [Lazaro et al. \(2014\)](#), vinasse is also toxic because it contains potassium, sulfate, phenolic components and melanoidin. After pre-treatment, vinasse was ready to be used as a substrate for bio-hydrogen production.

### 3.2 Bio-hydrogen Production

After a preliminary study, the production of bio-hydrogen using vinasse was done with various concentrations of 10,000-50,000 mg/l COD vinasse. The result was presented in Table 1 and Fig. 1. The highest Hydrogen Production Rate (HPR) achieved at the concentration of 50,000 mg/L (109.98 mL H<sub>2</sub>/mg COD/d), while the lowest HPR generated at concentration of 10,000 mg/L (12.76 mL H<sub>2</sub>/mg COD/d). At the concentration of 20,000-50,000 mg/L COD, bio-hydrogen gas volumes rise significantly from third day to ninth day, except concentration of 10,000 mg/L decline from third day to ninth day. There are many factors that affect the production of bio-hydrogen with a wide range of substrate concentration. According to [Buitrón and Carvajal \(2010\)](#) the substrate concentration of vinasse has two effect on the production of bio-hydrogen, (a) the substrate concentration can potentially become a barrier and (b) there are some concentrations that can maximize the production of bio-hydrogen. Which can

be a barrier of bio-hydrogen production from vinasse is the accumulation of organic acids that are generated when a process takes place and the presence of toxic components in vinasse like phenolic components and furfural. The research also indicates a difference of metabolic pathways that are prominent in every different vinasse concentration ([Lazaro et al., 2014](#)).

The concentration of 10,000 mg/L COD vinasse was obtained the lowest HPR of the entire treatment. From the Fig.1, it can be seen that bio-hydrogen gas was decline from third day to ninth day. The highest gas earned on third day and lowest on ninth day, it was suspected because of the carbon source on production of media has run out on third day, so all that's left was the toxic compounds like phenolic compounds and furfural ([Lazaro et al., 2014](#)). In addition, the decrease of bio-hydrogen gas can also be affected by the differences of COD concentration that affect metabolic pathways at each concentration so the results also vary. A low concentration of vinasse can affect the production of propionic acid. Furthermore, according to [Kim et al. \(2008\)](#), hydrogen is not produced if the by-product are the lactic acid and propionic acid. Levels of COD removal upon the concentration of 10,000 mg/L COD can be seen in Fig.2. The highest level of COD removal was on third day and the lowest on ninth day. COD removal rate at this concentrations was the lowest from all treatment. Efficiency substrates of this concentration highest on third day and lowest on ninth day (Table 2).

The result of Hydrogen Production Rate (HPR) of concentration 20,000 mg/L COD can be seen in Table 1 and Fig. 1 (36.63 mL H<sub>2</sub>/mg COD/d). The highest bio-hydrogen gas achieved in the sixth day and lowest on the ninth day. Bio-hydrogen gas in this concentration was higher than concentration of 10,000 mg/L. The levels of COD removal was assumed to be the maximum COD levels that can be used by *R. marinum* to produce bio-hydrogen at concentration 20,000 mg/L (Table 2, Fig.2). COD removal rate at this concentrations was higher than concentration of 10,000 mg/L. The concentration of 30,000 COD mg/L obtained HPR namely 71.65 mL H<sub>2</sub>/mg COD/d. Bio-hydrogen gas in this concentration has increased from third day to ninth day and higher than previous concentration. HPR and COD Removal Rate were also higher than previous concentration. The highest efficiency of substrate at this concentration was on the ninth day and lowest on third day. The concentration of 40,000 mg/L COD Vinasse has obtained HPR 99.23 mL H<sub>2</sub>/mg COD/d. HPR and COD removal rate this concentrations were higher than previous concentration. The concentration of 50,000 mg/L COD achieved the highest volume of bio-hydrogen gas, HPR and COD removal rate of the entire concentration (Table 1, 2 and Fig.1, 2). The efficiency of the substrate on this concentration is also the highest of the whole concentration. This was allegedly due to more levels of COD concentration were used then the more bio-hydrogen gas also produced. Having regard to the

**Table 2.** Result of Efficiency Substrate and COD Removal

COD Conc. (mg/L)	Days	Efficiency of Substrate (%)	COD Removal (mg/L)	COD Removal Rate (mg COD/L/d)
10,000	3	23.58	2120.00±47.1	159.33
	6	22.34	2020.00±94.2	
	9	17.44	1626.66±365.3	
20,000	3	14.56	2353.34±235.6	534.33
	6	17.73	3253.33±612.8	
	9	26.27	5043.33±624.6	
30,000	3	10.11	2786.67±23.5	815.44
	6	19.03	5686.66±612.8	
	9	24.75	7192.67±718.8	
40,000	3	12.17	4036.66±860.3	865.44
	6	19.07	6720.00±471.3	
	9	20.75	7760.00±282.8	
50,000	3	9.19	3586.67±412.2	1437.66
	6	14.86	6386.67±141.4	
	9	30.06	13443.33±954	

**Table 3.** Chemical Properties of Substrate after Production

COD Conc. (mg/L)	Days	Total Organic Acid (%)	pH
10,000	3	0.03	6.19
	6	0.19	6.01
	9	0.11	5.94
20,000	3	0.13	6.06
	6	0.2	5.94
	9	0.22	5.91
30,000	3	0.18	6.05
	6	0.26	5.95
	9	0.28	5.98
40,000	3	0.19	6.11
	6	0.37	6.03
	9	0.41	5.97
50,000	3	0.15	6.1
	6	0.25	6.16
	9	0.39	6.05

length of fermentation and other factors that affect the production of bio-hydrogen optimally. Some of the research that uses vinasse substrate for the production of bio-hydrogen were research of [Buitrón et al. \(2014\)](#), resulting HPR in 57.4 mL H<sub>2</sub>/L-h using substrate tequila vinasse with thermal pre-treat anaerobic sludge as an inoculum bio-hydrogen producer. The research of [Santos et al. \(2014\)](#) produced hydrogen effectively amounted to 0.79 mmol/g COD on substrate concentration vinasse of 30,000 mg COD/L.

### 3.3 Chemical Properties of Substrate after Production

The chemical properties showed that total organic acids substrate after production was reduced but still available. The total organic acids of vinasse was 11.15 mg/L, and reduces gradually. pH of substrates after pre-treatment was 8, but after productions decreased into range (5.91-6.19). This is due to study by [Budiyo et al. \(2013\)](#), the decrease of pH can be caused by the accumulation of VFA Production when vinasse is decomposed. When vinasse is decomposed into biogas, biogas will be produced without going through the hydrolysis phase but directly into acidogenesis phase. In the phase of acidogenesis, the short chain molecular component is converted into VFA.

## 4. CONCLUSIONS

The differences of COD concentration and the length of fermentation time have an effect on bio-hydrogen gas production and Hydrogen Production Rate (HPR). The highest levels of bio-hydrogen gas, HPR and COD Removal Rate were achieved at 50,000 mg/l COD. Thus it can be concluded that the concentration of COD Vinasse and the length of fermentation time have an effect on production of bio-hydrogen gas using *Rhodobium marinum*.

## 5. ACKNOWLEDGEMENT

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