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ORGANIC WASTE IN INTEGRATED BIO-CYCLES FARMING SYSTEM AS A SOURCE OF RENEWABLE ENERGY OF GAMA-BIO-HYDROGEN BY BACTERIA *Enterobacter aerogenes*

[1*]Cahyono Agus, [2]Eka Sari, [3]Nendyo A. Wibowo, [4]Aries B. Sasongko, [5]Handojo H. Nurjanto, [6]Dewi Wulandari

[1]Faculty of Forestry UGM, Bulaksumur, Yogyakarta, Indonesia

[2]Faculty of Biology UGM, Sekip, Yogyakarta, Indonesia

[3]Graduate School UGM Yogyakarta, Indonesia

[4]Faculty of Biology UGM, Sekip, Yogyakarta, Indonesia

[5]Faculty of Forestry UGM, Bulaksumur, Yogyakarta, Indonesia

[6]SEAMEO BIOTROP, Jl Raya Tajur km 6, Bogor, Indonesia

*Corresponding Author

ABSTRACT

All forms of energy is very costly, however, renewable energy generally may cheaper, while fossil fuels is mostly more expensive. Integrated Bio-cycle Farming System (IBFS) is an alternative system which harmoniously combines agricultural sectors, and non-agricultural aspects, on landscape ecological management. The cycle of energy, carbon, water, nutrient, production, crop, money was managed to be has multifunction and multi-product (Food, Feed, Fuel, Fiber, Fertilizer, Pharmacy, Edutainment, Eco-tourism). The purpose of this paper is to examine the growth of bacterial cells and bio-hydrogen production by Enterobacter aerogenes bacteria from organic waste of cajuput leaves. A stirred tank bioreactor that will be used is Fed Batch, an engineering process to produce maximal bio-hydrogen. Engineering process is used to increase the speed of the hydrogen production process and the acquisitions of hydrogen gas results to be more effective and efficient. Fermentation and production of bio-hydrogen was optimized after 24 hours, with the highest at a substrate concentration of 20%. The hydrogen production has positive correlation with the number of E. aerogenes bacteria through a dark fermentation method, a non-photosynthetic process that can do at all day without sunlight.

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Keywords: Enterobacter aerogenes; bio-hydrogen, integrated farming; organic waste; renewable energy

1. INTRODUCTION

All forms of energy are highly cost. However, renewable energy generally is getting cheaper, while fossil fuel generally is getting more expensive. Hydrogen is an important alternative future energy with socio-economic and environmental benefit. Hydrogen contain of very high energy per unit weight comparing to any known fuel (142 kJ/g) (Guwy et al., 2011), but very low energy for its volume. Hydrogen can be an alternative important energy carrier that can be obtained from biomass as well as decomposition of organic compounds (Balat, 2008).

Bio-hydrogen production is an alternative to the method for hydrogen gas production. Enterobacter aerogenes is representative a facultative anaerobe bacterium, well-known as one of a good producer of hydrogen (Dipasquale et al., 2012). This bacterium is described fermented most diverse substrate such as organic urban solid waste (Valdez-Vazquez et al., 2009). E. aerogenes can rapidly consume oxygen and recover the activity of Fe-hydrogenase under anoxic condition in contrast to strict anaerobes which are sensitive to oxygen inhibition (Kapdan and Kargi, 2006).

Integrated Bio-cycle Farming System (IBFS) was developed by KP4 UGM with more in-depth studies conducted through ICM (Integrated Crop Management), INM (Integrated Nutrient Management), IPM (Integrated Pest Management) and IMM (Integrated Moisture Management). IBFS has multifunction and multi-product (Food, Feed, Fuel, Fiber, Fertilizer, Pharmacy, Edutainment, Eco-tourism etc.) with technological strategy of 7R (Reduce, Reuse, Recycle, Refill, Replace, Repair and Replant). The system should collaborate and develop networking system between ABCG (Academic, Business, Community and Government) with economical-, environmental- and socio-cultural-approach as a characteristic of education for sustainable development (Agus et. al., 2011).

Although some studies have been done on the effect of E. aerogenes on hydrogen production from biomass (Kapdan and Kargi 2006; Valdez-Vazquez et al. 2009; Batista et al. 2014), however, information on optimization of substrate used and bacteria number in producing bio-hydrogen from organic agricultural waste is only little. Furthermore, fermentative hydrogen production mostly 59% is conducted by using pure monosaccharides, while sustainable feedstock such as using organic waste material is still low only 20% (Elsharnouby et al., 2013). The use of pure carbohydrate source is very expensive raw materials for real scale hydrogen production. The use of organic agricultural waste material in this study is a challenge for the future renewable energy of hydrogen production.

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In this study we selected and propagated E. aerogenes bacteria and modified its media growth with variation of substrate amount. We build an equipment to produce bio-hydrogen and separator for product purification. The optimum used of E. aerogenes is investigated by following question: (1) how long is the optimum time necessary to produce bio-hydrogen by E. aerogenes? (2) does number of effective E. aerogenes influence on the bio-hydrogen production? (3) does variation concentration of substrate affect the growth of E. aerogenes and its bio-hydrogen production ?

2. MATERIAL AND METHODS

2.1. Fed-batch fermentation experiment

Fed-batch dark fermentation experiment was performed in bio-reactor (Figure 1). The bioreactors had 2 liters volume with stirrer inside. About 500 ml starters were diluted to a volume of 1.5 L of sterilized water with addition of glucose and other nutrients according to the treatments. Fermentation was allowed for 72 hours, and continued by adding agricultural waste substrates with concentration 1%, 5%, 10% and 20%. Production of bio-hydrogen was analyzed every 6 hours within 48 hours. During fermentation, pH and temperature in the bioreactor were measured and maintained at optimum condition (pH 7 and temperature 37.5oC) and its effect on bio-hydrogen production was studied. To optimize the process of fermentation, we controlled the addition of substrate concentration, interval addition of substrate, addition of nutrients, fermentation time, stirring speed, temperature fermentation, and pH.

2.2. Preparation of substrate

Organic substrate used in this study was Amaranthus tricolor, A. hybridus, Alternanthera amoena, and Lactuca sativa. Approximately 400 g of each substrate were weighing and homogenized by adding deionized water into blender. Homogenized substrate was pour into ten replications of 500 ml Erlenmeyer. The substrate was sterilized by autoclave 121oC for 5 minutes.

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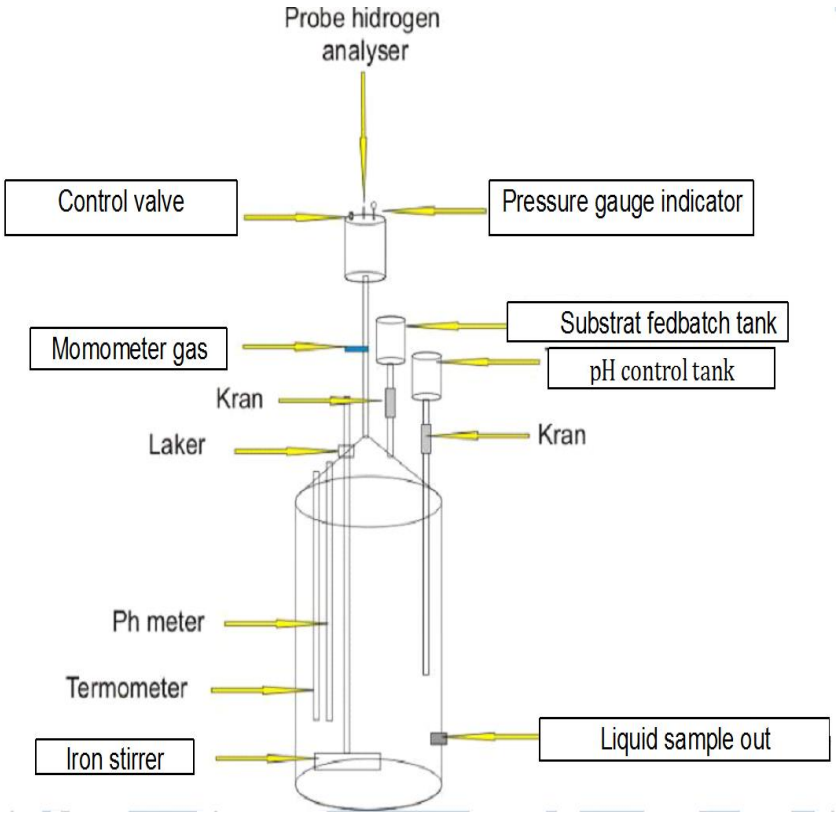

Figure 1. Design for FedBatch bioreactor used in this experiment

2.3. Culture of Enterobacter aerogenes bacteria

Isolate of E. aerogenes was cultured in agar media and incubated in 4oC under dark condition. Reculture of E. aerogenes was conducted by inoculated the colony into new agar media every one month. Inoculation of E. aerogenes was conducted under aseptic condition. Cultured of E. aerogenes was inoculated into 500 ml liquid media contained of 2% glucose, 2% yeast extract, 3.5 mg/L FeSO4, 5 g/L NaCl, 0.5 % protease peptone. Before inoculation of E. aerogenes, nitrogen gas was flowed into media to make anaerobe condition. The inoculated liquid media was incubated for 72 hours, followed by addition of sterilized organic agricultural waste substrate. This substrate was added in amount of 1% to 7% of liquid media volume and incubated it for one week to be starter media.

2.4. Analysis of bio-hydrogen

Bioreactors was prepared to have volume of 2 L and fitted with a stirrer. Starter media in 500 ml Erlenmeyer was diluted into a volume of 1.5 L. Organic agricultural waste substrate was added

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varied on the concentration of 1%, 5%, 10% and 20%. Production of bio-hydrogen was analyzed every 6 hours for 48 hours by calculating glucose concentrations and the number of bacteria cell. During the process of fermentation, temperature and pH in the bioreactor were maintained at 40oC and pH 5.5-6 by applying NaOH 4 M. Optimization of operating conditions was carried out for each variable: addition of substrate concentration, fermentation time, interval addition of substrate, addition of nutrients, stirring speed, temperature fermentation and pH control. Hydrogen gas production was analyzed by gas chromatography equipped with a thermal conductivity detector by using Argon and Helium as carrier gases. The cumulative H2 volume was calculated from H2 percentage and total volume of biogas production.

3. RESULT AND DISCUSSIONS

The number of bacterial cells of Enterobacter aerogenes increased drastically during the first 4 hours fermentation period of organic waste at level of 20 x 107 per ml for 1% and 5% substrate, and 25 x 107 per ml for 10% and 20% substrate of cajuput oil leaves (Figure 2). The amount of hydrogen production increased drastically during the first 24 hours fermentation period of organic waste by E. aerogenes at level of 10 mmol, and then was optimized after 24 hours.

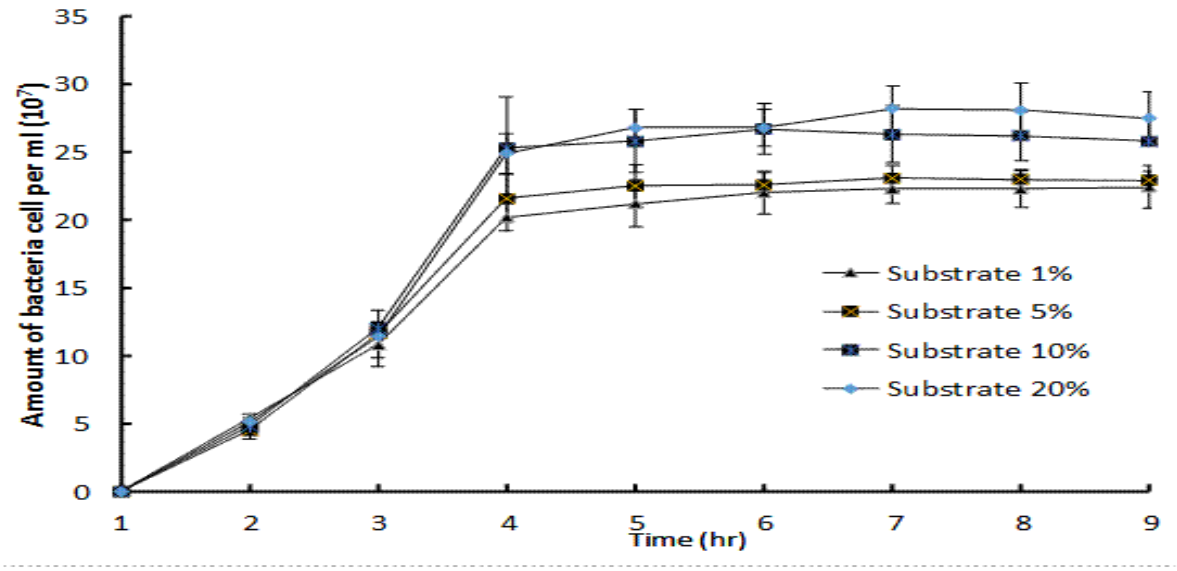

Figure 2. The growth of bacterial cells during fermentation

During early stage, the initial substrate concentration had less impact on hydrogen production that could be because of the adaptation period. The maximum hydrogen production was recorded when substrate addition was 20%. At higher substrate addition, fermentation process was still

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continued even after 48 hours. Corresponds to our result, high hydrogen production was noted when glucose concentration was higher (20 g/L) in 20 hour after fermented by *E. aerogenes* (Liu *et al.,* 2009). Comparing to another substrate source, i.a. glucose, substrate from organic waste material may be more effective in producing hydrogen. Previous studied showed that utilization efficiency of glucose as substrate was decreased by increasing glucose concentration (Liu *et al.,* 2009). It indicates that high concentration of glucose may inhibit the fermentation process. As it mentioned that one of some factors influence the fermentative hydrogen production is substrate (Wang and Wan, 2009). This result implied that the use of organic waste substrate material could be more effective and have more environmental beneficial impact than pure monosaccharides.

Table 1. Production of hydrogen from other references

Mechanisme	Substrate	Result	References
Photo-fermentation	7.5 mM malate acid	120 mL total	Eroglu, 1997
Photo-fermentation	Milk waste	85 mL total	Turkarslan, 1997
Photo-fermentation	60mL Na lactate	269 mL% total	Barbosa, 2000
Combination	40 mL glucose	52 mL total	Redwood, 2006
Photo-fermentation	28 mM glucose	5 mL H_2	Fang, 206
Photo-fermentation	30 mM glucose	70mL total	Li, 2007
Photo-fermentation	30 mM Na lactate	255.4 mL total	Li, 2007
Photo-fermentation	25 mM glucose	45 mL H_2	Current research
Photo-fermentation	50 mM glucose	120 mL H_2	Current research
Photo-fermentation	Sugar cane wase	50 mL H_2	Current research

Bio-hydrogen production through biological process can be done by biophotolysis, dark fermentation, and photo fermentation. Among three of them, dark fermentation is considered more environmental beneficial due to simultaneous waste treatment and hydrogen production (Liu *et al*., 2009). Furthermore, the use of dark fermentation method such in this study is more effective as it is faster in producing hydrogen, does not require light, which means can be conducted in all time, in comparing to another two biological process (Table 1). In addition to fermentation type, the media pH range from 5 to 6 in this study gave optimum condition to cultivate hydrogen production under anaerob condition (Tanisho, 1998).

Fermentation was optimized started 24 hours after incubation by measuring hydrogen production (Figure 3). This hydrogen production continued to be increased by the length of incubation time. The hydrogen production was highest in the addition of 20% substrate. This result indicates that higher contain of substrate may stimulate to higher growth of E. aerogenes for their metabolic activity. The growth of bacteria was showed started high in 24 hours after fermentation with the

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highest substrate 20%. The more substrate here is the carbon source for E. aerogenes to grow. Furthermore, the fermentation temperature 40°C in this study gave optimum condition for the E. aerogenes to grow (Tanisho 1998). A positive correlation between number of bacterial cell and rate of hydrogen production was observed. The hydrogen production was obtained at a substrate concentration of 20%. This occurs because more substrate will increase the metabolic activity of bacterial cells, facultative anaerobic and hydrogen generated by the activity of E. aerogenes.

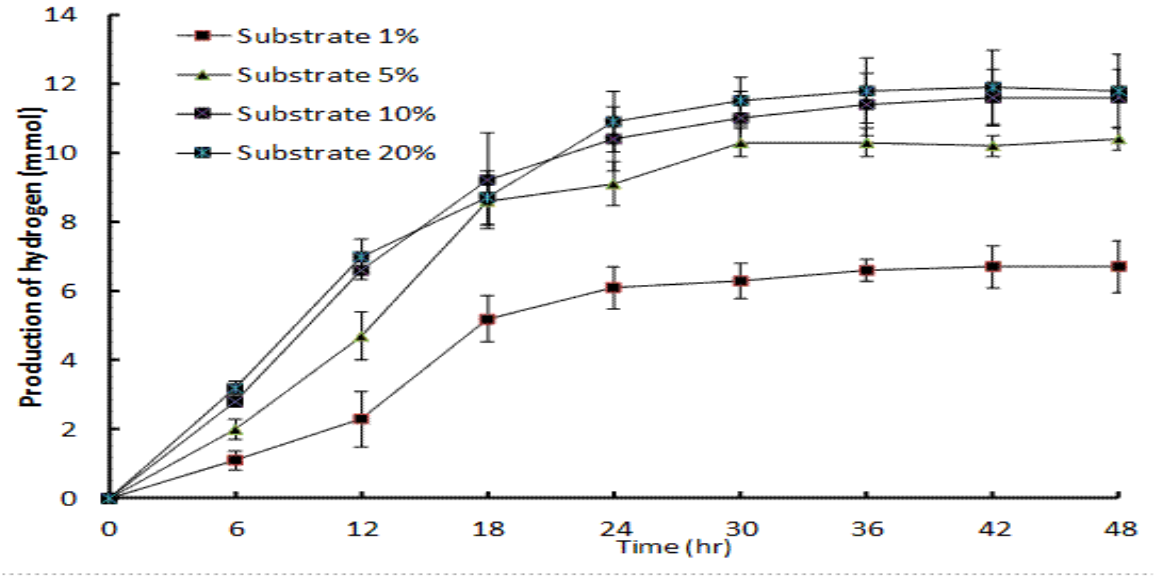

Figure 3. Total hydrogen produced during fermentation

E. aerogens fermented lactose with the final product of organic acids such as acetic acid, lactic acid, butyric acid and alcohols such as 2,3- butanediol and ethanol (Rachman *et al*., 1997). This facultative anaerobic bacteria and chemo-organotroph may growth optimum in temperature at 30-37°C. E. *aerogenes* can live in a fairly broad pH range. Isolation of *E aerogenes* HU-101 from methane were grown in facultative anaerobic conditions at 37°C with 2% glucose content in a complex medium has reached the stationary phase at 24 hours, a very short lag phase Yokoi, *et al*., 1997).

Bacteria of *E. aerogenes,* fermented lactose with the final product in organic acids such as acetic acid, lactic acid, butyric acid and alcohols such as 2,3- butanediol and ethanol. This facultative anaerobic bacteria and chemo-organotroph with optimum growth temperature of 30-37°C can live in a fairly broad pH range. Yokoi *et al.* (1997*)* reported that *E aerogenes* isolated from acid uric HO-39 can live at pH 3.3 in aerobic and pH 4 in anaerobic media. Rachman *et al*. (1997) isolated *E aerogenes* HU-101 from methane, could be grown in facultative anaerobic conditions

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at 37 ° C with 2 % glucose content in a complex medium has reached the stationary phase at 24 hours, a very short lag phase.

Organic waste with concentration at 1% got their optimum productivity at level of 6 mmol, whereas that of 5%, 10% and 20% got it at 10-12 mmol (Figure 3). The results showed that production of hydrogen is linear to the amount of bacteria cells Hydrogen production through non-photosynthetic process has several advantages, among others, does not require sunlight so it can last all day (Liu *et al.*, 2009). Hydrogen production through non-photosynthetic process has several advantages, among others, does not require sunlight so it can last all day (Table 1).

It is reported that *E. aerogenes* VP-1 reached the stationary phase at 5 hours, while VP-2 *E. aerogenes* reached stationary phase at 6 hours (Ito *et al*., 2005). *E. aerogenes* mutant AY-2 were grown in facultative anaerobic conditions at 37°C with 2% glucose content in the medium of the complex has a long lag phase is 4 hours . The lag phase is much longer than in wild-type phase lag is less than 2 hours. Growth of *E. aerogenes* AY-2 not reach stationary phase at 24 hours yet, while wild-type stationary phases have undergone (Rachman *et al*., 1997).

CONCLUSION

Fermentation and production of bio-hydrogen was optimized after 24 hours, with the highest at a substrate concentration of 20%. The production of hydrogen has positive correlation with the number of *E. aerogenes* under dark fermentation method, a non-photosynthetic process that can be done at all day without sunlight.

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