Performance of continuous stirred tank reactor (CSTR) on fermentative biohydrogen production from melon waste

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Abstract. This research was meant to investigate performance of continuous stirred tank reactor (CSTR) as bioreactor for producing biohydrogen from melon waste through dark fermentation method. Melon waste are commonly generated from agricultural processing stages i.e. cultivation, post-harvesting, industrial processing, and transportation. It accounted for more than 50% of total harvested fruit. Feedstock of melon waste was fed regularly to CSTR according to organic loading rate at value 1.2 – 3.6 g VS/ (l.d). Optimum condition was achieved at OLR 2.4 g VS/ (l.d) with the highest total gas volume 196 ml STP. Implication of higher OLR value is reduction of total gas volume due to accumulation of acids (pH 4.0), and lower substrate volatile solid removal. In summary, application of this method might valorize melon waste and generates renewable energy sources.

1. Introduction
Hydrogen is the ultimate choice of energy carrier in future due to its superior properties. It emits less greenhouses gases, more energy efficient conversion up to 50%, higher energy densities, and it is renewable [1]. It can easily be produced from water electrolysis when there is sufficient free excess of electricity such as one from geothermal power plant. However, lack of excess of electricity leads to avoid the use of electrolysis method [2]. There is more promising method to produce hydrogen from cheap raw materials i.e. dark fermentation using organic waste as raw materials [3].

Fermentative production of hydrogen using the waste empowers microbial community to metabolize organic matter into volatile fatty acids. Simultaneously, it emits electron to hydrogenase converting proton into hydrogen (H₂). This method is influenced by many factors e.g. temperature, pH, substrate concentration, inhibitors, and reactor types such as continuous stirred tank reactor, plug flow reactor, fluidized bed reactor [4].

Continuous stirred tank reactor (CSTR) is widely used as means to convert reactants into valuable products in chemical industries. It is used commonly for liquid or suspension reaction yet rarely occupied as means for gas phase reaction. The fermentation of organic waste into renewable biohydrogen occurs in three phases i.e. liquid (diluted water), solid (total solid of melon waste) and gas phase (hydrogen and CO₂) [5]. Controlling optimum condition i.e. gas yield, substrate reduction, pH, inhibitory chemical concentration of the fermentation is challenging tasks on three phases CSTR. Moreover, change of organic loading rate (OLR) can cause significant interruption of CSTR stability performance. When OLR is high, there is tendency of accumulation of acids and inhibitory compounds leading to lower gas production and yield. On the other hand, lower OLR may reduce the unstable of CSTR due to sufficient ratio between source of microbial food (substrates) and certain population of microorganisms. Lower OLR also prevents accumulation of volatile fatty acids which maintain environmental condition at optimum pH level (pH 4 – 6). It is therefore important to study the performance of CSTR on biohydrogen production from organic waste.
Melon (*Cucumis melo*) is one of national fruit fresh trading commodities throughout Indonesia. According to National Statistics Bureau of Indonesia, there was more than 150,000 ton of harvested melon in 2014, which some portion turned into waste approximately 10 – 50%wt due to deterioration, physical damage or else [6]. This melon waste has been widely treated as organic waste through open dumping method. It causes environmental problems such as methane emission, water contamination, bad odor, and eventually global warming. To mitigate such effects, it was proposed to utilize this melon waste as raw material to produce biohydrogen as renewable energy.

Melon (*Cucumis melo*) contains 92 – 94%wt moisture, 6 – 8% dry matter (total solid), 6 g/ 100 g of fresh melon [7]. Melon seeds contains 52.3% of testa and 47.7% of kernel, oil 22.1 – 53.5%, 21.8% of crude protein [8]. Eugenol is major constituent in skin of pocket melon i.e.15.3% which is known also as anti-microbial agent [9], [10].

Therefore, this research was meant to investigate the performance of CSTR in fermentation of melon waste to produce hydrogen.

2. Materials and methods
   2.1. Feedstocks
   Orange and melon fruit waste was collected from local fruit market in Yogyakarta. The two were peeled and cut off, followed with grinding using kitchen blender, thereafter was called feedstock. It was stored at freezer (-5°C) prior to be used as feed to CSTR.

   2.2. Inoculums
   Hydrogen producing microorganisms was originally from residual sludge of cow dung biogas plant which had been treated through pH 5.0 (24 hours) and heat-shock treatment (95°C, 45 min).

   2.3. Experimental Procedures
   CSTR was initially filled with inoculums, substrates, and water for 3 days prior to feed with 1.2 g VS/ (l.d) organic loading rate. On the 4th day, quantity of 100 gr feedstock was fed to CSTR per day for 2 days followed with 1.8; 2.4; 3.0 and 3.6 g VS/ (l.d).

   2.4. Analysis
   Gas content was analyzed using Gas Chromatography Shimadzu 8A which equipped with MS 5A column and Thermal Conductivity Detector. It is run with Helium as gas carrier at 40 ml/min. temperature of oven, injector and detector was set at 60°C, 80°C and 80°C respectively. TS, VS and ash were measured based on standard method of APHA.

3. Results and discussion
   Continuous stirred tank reactor (CSTR) was used as bioreactor for fermentative biohydrogen production from melon waste. The performance of CSTR was noted in term of total volume gas produced, volatile solid reduction (VS removal), and profile of pH evolution. These performance parameters are presented in Figure 1-3 respectively.

   Figure 1 shows profile of total gas volume evolution during fermentation period with various value of organic loading rate. At initial stage, CSTR was loaded with organic loading rate value at 1.8 g VS/ (l.d) for period of 12 days. Gas production rate was significantly high at this stage. It was recorded that this fluctuate production achieved the highest total daily gas production 196 ml STP. At the beginning, total gas volume was high since there is sufficient microorganism to consume fresh feed of melon waste. On the next days, total gas volume decreased gradually due to accumulation of total organic solid which was not degraded during this period. On the following organic loading rate value at 2.4 g VS/ (l.d), the total gas volume was lower compare to the first period. The highest total gas production was 176 ml STP. It can be seen that there is no significant different between these two OLR values toward gas production. When OLR increase to 3.0 or 3.6 g VS/ (l.d), total gas volume decreased significant and consistent to value lower than 100 ml STP. On the day 40th – 42nd, there was no
withdrawal of gas due to technical reactor problem, so that the produced gas was collected and measured on the day 43rd. This total cumulated gas was significantly high up to 447 ml STP/3 days (average 147 ml STP/day). It can be concluded that OLR value lower than 2.4 g VS/ (l.d) is better compare to higher value more than 3.0 g VS/ (l.d).

Figure 1. Effect of organic loading rate toward total volumetric gas production

Figure 2 shows value of volatile solid (VS) percentage of melon waste inlet and outlet of CSTR. At initial stage of OLR 1.8 g VS/ (l.d), the VS outlet of melon waste was 65 – 85%wt. The lowest value of VS outlet correspond to higher gas production indicating that more substrat was consumed and degraded by microorganism to produce volatile fatty acids and hydrogen. In the second period of OLR 2.4 g VS/ (l.d), the VS outlet slightly higher compare to the first one. Therefore, it implies that there was lower gas production as seen in Figure 1.
Figure 2 shows the evolution of pH during each period of organic loading rate. At initial stage, pH of fermentation system was optimum at condition between 5 – 7. It indicates that there was no significant accumulation of volatile fatty acids during the first period OLR 1.8 g VS/ (l.d). However, increasing OLR to 2.4 g VS/ (l.d) was able to disturb the pH stability to value pH 4.0 or even further lower. This acidic condition occurred due to accumulation of volatile fatty acid which was generated from substrate degradation. Microorganisms was not able to sustain their metabolism of degradation of organic matter content in melon fruit waste.

Hydrogen production through dark fermentation is accomplished via complex microbial reaction. However, it is simplified in following reaction (equation 1 – 2). When acetate is the only volatile fatty acid products, maximum hydrogen gas production is achieved with yield 4 mol H$_2$/mol glucose or equivalent to 498 ml STP H$_2$/gram glucose. When butyric acids is the product, hydrogen yield become less equal to half of the first reaction [11].

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C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 4H_2 + 2CO_2 \quad (1)
\]

\[
C_6H_{12}O_6 + 2H_2O \rightarrow 2(CH_2)_3COOH + 2H_2 + 2CO_2 \quad (2)
\]

Previous experiments on biohydrogen through dark fermentation using various type of substrates have been reported with wide range of practical hydrogen yield. For instance, utilization of peach pulp as substrate was reported with the highest yield 123.27 ml H$_2$/gTOC and production rate 35.6 ml H$_2$/h [12]. Mesophilic dark fermentation using novel strain Clostridium sp YM1 and glucose solution was conducted with hydrogen yield 1.7 mol H$_2$/mol glucose (equivalent to 211 ml STP H$_2$/gram glucose) [13]. Fermentation of food waste to produce hydrogen was also carried out in continuous stirred tank reactor with hydrogen yield 261 ml H$_2$/g VSadded and 379 ml H$_2$/(l.d) (38.6%v) [14]. In comparison, this experiment was carried out with the highest hydrogen yield and production rate 2.2 ml STP H$_2$/g VSadded (8%v H$_2$) and 49 ml/(l.d). This lower results was suspected due to pH descending evolution during fermentation (Figure 3). This pH decrease occurs because the CSTR was not designed with pH control and there was no addition of buffer solution, it was expected to maintain simplicity of reactor. In addition, accumulation of eugenol which acts as antimicrobial agents might also inhibits microbial metabolisms to produce high yield of hydrogen.
4. Conclusion
Performance of biohydrogen production in CSTR was highly affected by the organic loading rate of melon waste. Optimum condition of OLR was achieved at value 2.4 g VS/(l.d) with the highest total gas volume 196 ml STP. At higher OLR value, there is tendency of acid accumulation leading to lower gas production and substrate volatile solid removal. pH control system and addition of buffer solution might improve hydrogen production.

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