The Influence of Hydrodinamic Factor on Fermentative Hydrogen Production Process In Stirred Tank Reactor

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Abstract

Hydrogen has been recognized as an ideal and clean energy source. It is not only a renewable energy source but also environmentally benign with water as the only reaction product. In this research, hydrogen was produced using glucose as substrate and Enterobacter aerogenes (NBRC 13534) as microorganism. The purpose of this research is to improve the efficiency of fermentation through hydrodynamics factor setting. Analysis of hydrodynamics in the multiphase system is urgently needed in order to provide the optimal performance. Hydrodynamics factor is suspected to play a role in determining the quality of ingredients mixed in the reactor and releasing hydrogen gas from the mixture, therefore hydrodynamics is necessary also to determine scale-up parameters of the reactor that used in commercial production. The reactor was employed for batch fermentation with a working volume of 5 l. It also was supplemented with 45^0 6-blades pitched blade turbine impeller (stirred at 46 rpm; 81 rpm; 116 rpm; 165 rpm) to investigate the effect of rate of stirring on biohydrogen yield, glucose concentration and number of cells. Operation condition was kept on 37 ^oC and pH 5.5 – 6.5. The gas product (mainly H_2 and CO_2) were analyzed by GC-TCD, glucose concentration was analyzed by spectrophotometer and number of cells was analyzed using counting chamber method against absorbance of spectrophotometer. The results show that the enhancement of stirring speed can give a significant effect on the increasing of hydrogen yield, increasing number of bacteria and degradation of glucose concentration during fermentation process. The highest hydrogen yield is 0.107 mol H_2/mol glucose reacted at the stirring speed of 165 rpm.

1. Introduction

Energy is a vital need of humans in the world. Nowadays, energy suplay mainly depend on fossil fuels but in fact, it contributes to global environment and health problems. Therefore, alternative energy is needed to replace the fossil hydrocarbon sources. Among the new paradigm of sustainable energy, hydrogen is considered a viable alternative fuel for mobile and an ideal energy carrier.

H_2 has been recognized as an ideal and clean energy source. It is not only a renewable energy source but also environmentally benign. Hydrogen produces only water instead of greenhouse gases when combusted, generating a higher energy yield (122 kJ/g) by 2.75 times than hydrocarbon fuels (Kim et al., 2006). The current commercialized H_2 production processes, mostly hysicocemical process, include steam reforming-natural gas, electolysis, partial oxidation of fuel oil, gasification of coal, gasification of biomass and so on. These processes require external energy sources, so these are uneconomic (Kim et al., 2004). Biological production of hydrogen using microorganism is an exciting new area of technology development that offers the possibility to use a wide variety of low-price renewable feedstock and a suitable improvement of organic waste refuses treatments.

Biological hydrogen production processes can be classified to *photo* and *dark fermentation* processes. Dark fermentation, by anaerobic microorganisms produces hydrogen from a general anaerobic metabolism. The anaerobic bio-hydrogen production process is not only stable, but also fast in the production of H_2 compared to the photo-fermentation process (Kim et al., 2004).

Carbohydrates are the preferred substrate for hydrogen producing fermentations. Glucose, isomers of hexoses, or polymers in the form of starch or cellulose, yield different amounts of H_2 per mole of glucose, depending on the fermentation pathway and end products. When acetic acid is the end product, a theoretical maximum of 4 mole H_2 per mole of glucose is obtained:

$$C_6H_{12}O_6 + 2H_2O \longrightarrow 2CH_3COOH + 4H_2 + 2CO_2$$

When butyrate is the end product, a theoretical maximum of 2 moles H_2 per mole of glucose is obtained (Levin et al., 2004):

$$C_6H_{12}O_6 + 2H_2O \longrightarrow 2CH_2CH_2CH_2COOH + 2H_2 + 2CO_2$$

Bacteria known to produce hydrogen by anaerob nonphotosynthetic consist of obligate anaerobes and facultative anaerobes. *Clostridium butyricum* (Kapdan and Kargi, 2006), *Clostridium pasteurianum* (Liu and Shen , 2004), *Clostridium paraputrificum* M-21 (Evvyernie et al., 2001) dan *Clostridium bifermentants* (Wang et al., 2003) are obligate anaerobes microorganisms as a hydrogen-producing spore-forming. These microorganisms are very sensitive to oxygen and their hydrogen producing activities are completely inhibited by the presence of a very slight amount of oxygen in a feeding medium. In contrast, since facultative anaerobes consume oxygen rapidly, an anaerobic condition in a reactor is recovered immediately (Yokoi et al., 1995). Facultative anaerobes microorganism that mostly used to produce hydrogen is *Enterobacter aerogenes*. Hidrogen production by *Enterobacter aerogenes* is faster than photosynthetic microorganisms and it can survive where H_2 is abundant (Mahyudin, 2009).

Hydrogen production process involves multiphase (solid-liquid-gas) systems which homogeneity is one of the parameters to ensure maximum distribution of each phase. The rising of gases bubbles are responsible for the principal agitation in the system, during their ascent they induce turbulence, which leads cells and substrates in a random way, thus favouring homogeneity (Roza et al., 2002). But when the gases released were small, the agitation caused by the rising of gases bubbles would be insufficient to achieve homogeneity. Thus, mechanical agitation is needed to increasing the reactor performance.

Many researchers have investigated the influence of stirring speed on hydrogen production biologically (Chou et al., 2008, Gómez et al., 2009, Aceves-Lara et al., 2008), but the effort to study the influence of hydrodynamic factor (i.e. the influence of stirring speed) in the hydrogen production process in the stirred tank using 45^0 6-blades pitched blade turbine impeller have never been investigated. In this study, the influence of stirring on hydrogen yield, glucose concentration and number of cells was elucidated.

2. Experimental

Enterobacter aerogenes NBRC 13534 (produced in Hirayasu Ogino Lab., Osaka Prefecture Univ., Japan) was grown under anaerobic conditions on the culture medium containing the following components: glucose 2% (w/v), yeast extract 0.5% (w/v), $FeSO_4.7H_2O$ 0.15 g/l and distilled water. The reactor was employed for batch fermentation with a working volume of 5 l and the fermentation process occurred during 48 hours. Operational condition was kept on 37 ^oC and pH 5.5 – 6.5 by adding the 4 N NaOH soluion. It also was equiped with pumping down 45^0 6-blades pitched blade turbine impeller (stirring speed at 46; 81; 116; and 165 rpm) which the geometry of the reactor and impeller are shown in figure 1.

The gas product (mainly H_2 and CO_2) were analyzed by Gas hromatography with Thermal Conductivity Detector (GC-9A Shimadzu, Pack Column : Moleculer sieve 13X), glucose concentration was analyzed by spectrophotometer (Cecil Instrument CE 1011) using the MSDS *3,5-Dinitrosalicylic acid* D0550 reagent, and number of cells was analyzed using counting chamber method against the absorbance of spectrophotometer.

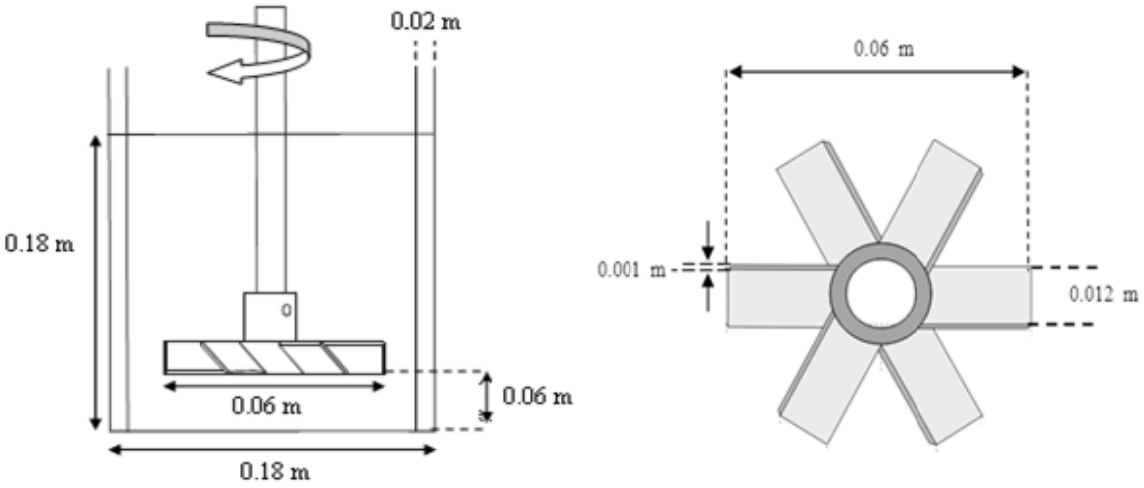
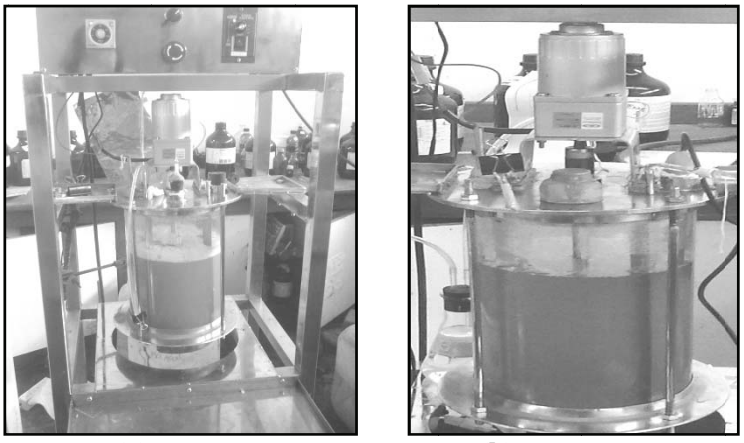


Figure 1: Geometry of the Reactor and Impeller

3. Results and Discussion

The influence of stirring on hydrogen production was investigated. Figure 2 shows the results of a batch culture carried out in a fermentor with stirring and without stirring. It is clear that when the fermentation was conducted without stirring, the gas produced during fermentation process was trapped inn the foam on top of liquid phase and can not release to gas phase. Observation for five days, the gas is still accumulated in the foam. Therefore, an effort is needed to breakdown the foam, thus the gas can release to the gas phase and leave the reactor. This observation shows that hydrodinamics factor is important on hydrogen production process. Stirring has a beneficial effect on reactor performance by improving mixing and decreasing H_2 partial pressure in the liquid phase. Generally, it is known that high H_2 partial pressure has a negative effect on H_2 production by decreasing the activity of hydrogenase and making the H_2 producton reaction thermodynamically unfavorable (Kim IS. et al., 2004).



(a) **(b)**

Figure 2: Substrat Condition without Stirring (a) and with Stirring (b)

Figure 3 illustrate the H_2 yield under various stirring speed. In figure 3, H_2 production clearly influenced by stirring speed where the highest H_2 yield was achieved at stirring speed of 165 rpm. Stirring speed corresponds to the rate of diffusion of a substrate across a biolayer based on Fick's first law (Chou et al., 2008) and decrease lag periode. During lag period, the bacterial cells modified their physiological state to take advantage of their environment and began multiplying and the spore suspensions develop into vegetative cells. At 41 rpm, H_2 gas was produced after 6 hours fermentation. In contrast, H_2 can be produced at early fermentation process when the stirring speed increased as shown in figure 4. The increasing of stirring speed causes the microorganisms consume the substrate

and release the gases faster and it require more time for complete fermentation. It can be seen that at 41 rpm, the fermentation process stopped before 30 hours fermentation but it was longer if the stirring speed increased. The increaing speedof impeller rotation makes the extention of the region of circultion flow. The top of circulation flow that arises from the bottom of the vessel becomes higher and reach the liquid surface. This flow pattern can break up the foam layer and the product gas can release from the liquid phase easier.

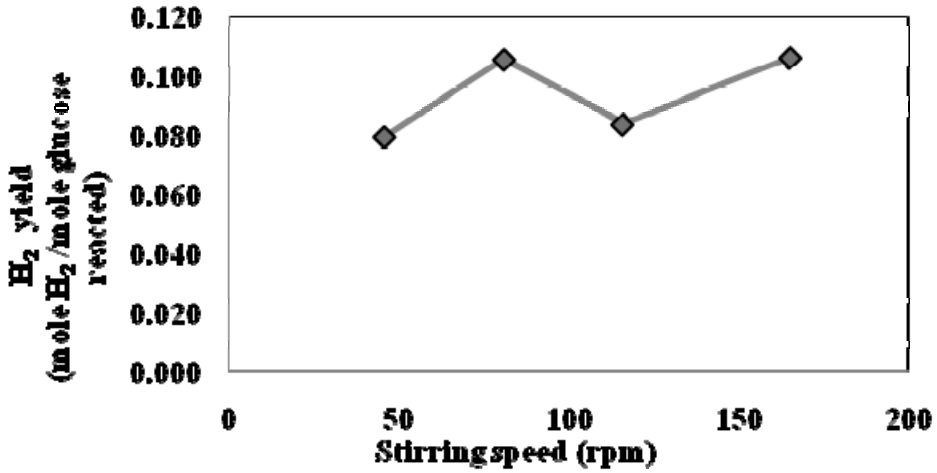


Figure 3: The relationship between H_2 yield and impeller speed.

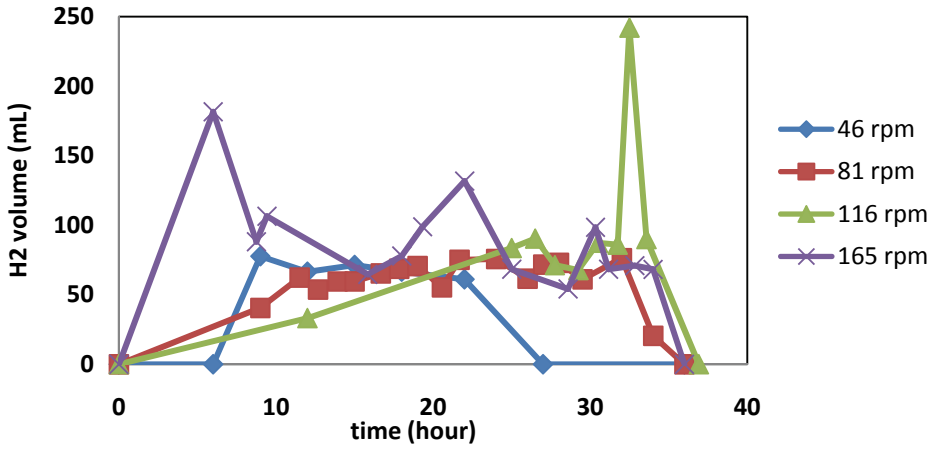


Figure 4: Production rate of H_2 gas under various impeller speeds

Hydrodinamics has a beneficial effect on the degradation of glucose concentration during fermentation process. As describe before that stirring speed can increasing the rate of diffusion of substrate across a biolayer and then enter into the cells. Eventhough as shown on figure 5, at early fermentation process, the degradation of glucose concentration at 81 rpm is slower than at 46 rpm. But in general, it indicate that stirring speed also could enhance the rate of glucose consumed by microorganisms and converted to products, i.e. hydrogen.

Figure 6 shows the number of cells under various stirring speeds. Comparing individual curve in figure 6, reflects that a high number of cells has occurred at a stirring speed of 165 rpm. These number of cells increased while the stirring speed increased. It should be noted that stirring speed also influence the enhancement of number of cells. If number of cells increase, hidrogen yield also increase.

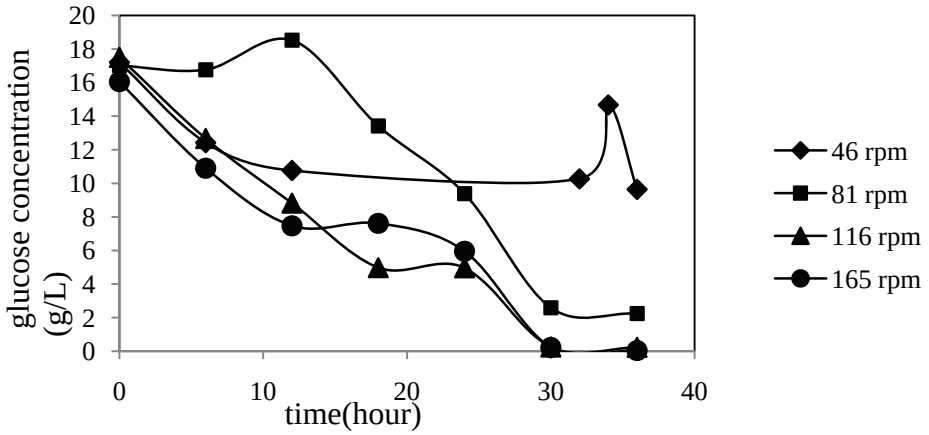


Figure 5: Degradation of Glucose Concentration during Fermentation Process Under Various Stirring Speeds

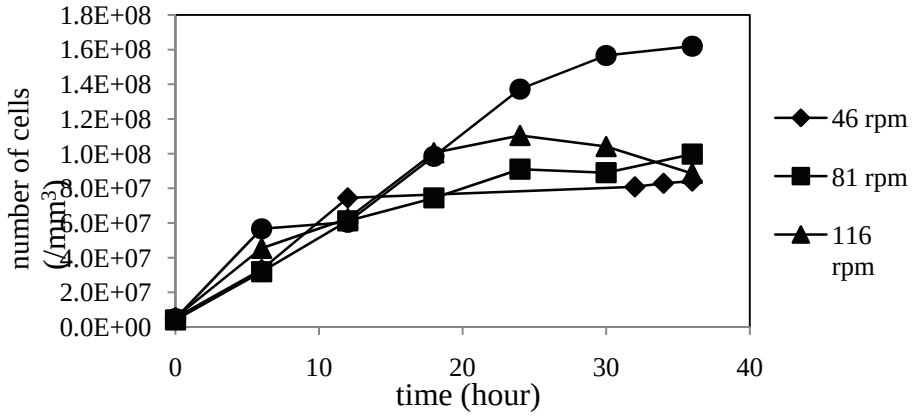


Figure 6: Number of Cells Under Various Stirring Speeds

4. Conclusion

The stirring gives significant effect to release the hydrogen gas from the substrate during the fermentation process. The reqion of circulaion flow that can reach the liquid surface can break up the foam layer and enhance the releaement of proct gas from the liquid phase. This effect can be enhancement by increasing the stirring speed that give higher hydrogen yield, increasing number of cell bacteria and degradation of glucose concentration. The highest hydrogen yield is 1.107 mol H_2/mol glucose at the stirring speed 165 rpm.

Acknowledgement(s)

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