

Original Research Paper

# Carbon Dioxide Utilization for an Enhanced Biohydrogen Production of a Biomass Hydrolysate

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**Abstract:** Bio-hydrogen (H<sub>2</sub>) production in a thermophilic anaerobic bioreactor with carbon dioxide (CO<sub>2</sub>) utilization at the headspace was investigated at different ratio of Biomass substrate to Microorganism (B/M). A 3 mL volume of 80% NaOH (m/v) solution was continuously hung at the headspace of the bioreactors to capture carbon dioxide. The utilization of CO<sub>2</sub> at its headspace, proved to enhance bio H<sub>2</sub> production. The highest Cumulative Biohydrogen Yield (CHY) of 131.81±3.47 mL-H<sub>2</sub>/gVS was measured at the ratio of 8.10 parts of biomass to 0.90 parts of Acclimatized Seed Sludge (ASS), B/M 9, while 4.17 parts of similar biomass to 0.83 parts of ASS (B/M 5) had a biohydrogen production of 90.70±16.67 mL-H<sub>2</sub>/gVS. The B/M of 6.12 parts of biomass to 0.88 parts of ASS (B/M 7) produced a CHY of 84.72±18.35 mL-H<sub>2</sub>/gVS while the control bioreactors without CO<sub>2</sub> utilization (without and with biomass substrate) yielded a 0.06±0.035 mL-H<sub>2</sub>/gVS and a 3.27±0.78 mL-H<sub>2</sub>/gVS respectively. The mechanism of the biofermentation in this anaerobic reaction produced two possible resulting reaction; the acetogenesis of CO<sub>2</sub> with H<sub>2</sub> and the hydrogenotrophic methanogenesis. These reactions consumes hydrogen in the process to produce methane or acids. The presence of 80% (m/v) NaOH solution at the headspace inside the bioreactors, utilizes the CO<sub>2</sub> producing a hydrogen-rich region in space: The highest average H<sub>2</sub> yield of 51.83 mL-H<sub>2</sub> after 49.1 h with B/M 9 without methane was due to carbon dioxide utilization in the bioreactors. A univariate ANOVA and Pair-wise Tukey HSD statistical analysis revealed that the CHY of B/M9 was significantly higher than the other B/Ms. The highest yield, 55.85 mL-H<sub>2</sub>/gVS obtained with the bioreactor of B/M 9 was optimum for H<sub>2</sub> production. The results concluded that H<sub>2</sub> production is also enhanced by CO<sub>2</sub> utilization at the headspace.

**Keywords:** Anaerobic Fermentation, Hydrogen Production, CO<sub>2</sub> Capture, Thermophilic Condition

## Introduction

Hydrogen (H<sub>2</sub>) is a promising alternative to fossil fuels due to its clean and high energy yield (122 kJ/g) and produces only water on combustion, It is environmentally friendly and is extensively used in a wide range of energy and industrial applications (Mizuno *et al.*, 2000; Rifkin, 2002). However, approximately 96% of the hydrogen consumed comes from steam reforming of fossil fuels, such as natural gas,

fuel oil and coal (Elam *et al.*, 2003; Ewan and Allen, 2005). Since the physicochemical H<sub>2</sub> production processes are energy-intensive and hazardous to the environment, there have been urgent needs for biological H<sub>2</sub> production from renewable materials. Though thermo-chemical methods of producing H<sub>2</sub> are an easier approach, biological methods are safer and economically viable (Kim *et al.*, 2008).

Hydrogen production from biomass wastes offers a dual benefit for controlling environmental pollution

while producing an alternative energy source. Hydrogen can be biologically produced through biophotolysis, light fermentation and dark fermentation (Nandi and Sengupta, 1998; Nath and Das, 2004). Despite the relatively lower yields of H<sub>2</sub>, dark fermentation is generally considered as the most promising method due to its higher H<sub>2</sub> production rate in the absence of any light source as well as the variety of carbon sources as substrates. Moreover, heterotrophic fermentative microorganisms exhibit relatively high growth rates and did not show any O<sub>2</sub>-limitation problem (Hallenbeck and Benemann, 2002; Bai *et al.*, 2004). Most of the studies on H<sub>2</sub> production are carbohydrate-based (Bai *et al.*, 2004; Ding *et al.*, 2008; Lin and Lay, 2004; Roychaudhury *et al.*, 1988; Zhang *et al.*, 2003) since these materials can be improved by inhibition of the activity of H<sub>2</sub>-quenching pathways or by reduction of H<sub>2</sub> and CO<sub>2</sub> partial pressure (Nath and Das, 2004; Ewan and Allen, 2005; Kim *et al.*, 2008; Nandi and Sengupta, 1998; Hallenbeck and Benemann, 2002; Bai *et al.*, 2004; Ding *et al.*, 2008; Lin and Lay, 2004; Roychaudhury *et al.*, 1988; Zhang *et al.*, 2003; Das and Veziroglu, 2001; Lamed *et al.*, 1988; Oh *et al.*, 2003; Park *et al.*, 2005; Kim *et al.*, 2004).

Cooked rice as a biomass waste is normally found in rice-eating countries. Its use as a substrate for H<sub>2</sub> production has been the subject of interest for years because of its carbohydrate-rich content. On the other hand, previous studies on the condition of the inoculums have also been found to affect the amount of H<sub>2</sub> production. Heat treatments of the inoculums were found to produce greater H<sub>2</sub> yields (57-72%) than with non-heat-treated inoculums (Oh *et al.*, 2003). Similarly, the amount of H<sub>2</sub> production from the thermophilic acidogenic culture condition was found to be higher than the mesophilic culture at all tested pH because of its methane-free environment and negligible propionate production (Zhang *et al.*, 2003; Kim *et al.*, 2004). Another condition to maximize H<sub>2</sub> production and reduce its losses via acetogenesis is to use a chemical scavenger to reduce the carbon dioxide (CO<sub>2</sub>) concentrations in the headspace of the bioreactors (Park *et al.*, 2005; Stolaroff *et al.*, 2008).

During fermentative H<sub>2</sub> production by mixed cultures, H<sub>2</sub> loss occurs through interspecies transfer (primarily to methane) and therefore, must be prevented. Based on a previous investigation, on biohydrogen production by dark fermentation from organic wastes and residues (Liu, D., Ph.D. thesis, Department of Environmental Engineering, Technical University of Denmark, 2008), the use of pH and temperature control as well as agitation seems to be the most applicable methods to prevent methanogenesis in an industrial-scale system. The highest H<sub>2</sub> production was found at neutral pH (pH 7). Furthermore, previous studies have also

demonstrated that bioreactors running at low HRTs presented a better performance in terms of H<sub>2</sub> production (Zhang *et al.*, 2003; Chen *et al.*, 2006).

Anaerobic fermentation is one of the best biotechnologies for H<sub>2</sub> production and various fermentation conditions had been optimized. One of the approaches is the sequestration of CO<sub>2</sub> at the headspace of the bioreactor to reduce H<sub>2</sub> losses via acetogenesis by using KOH (Park *et al.*, 2005). The CO<sub>2</sub> capture at the headspace resulted to H<sub>2</sub> production of 43%, from 1.4 to 2.0 mol of H<sub>2</sub>/mol of glucose (Park *et al.*, 2005). Another fermentation condition to maximize H<sub>2</sub> production is to control the Food to Microorganism (F/M) ratio. The F/M ratios between 7 and 10 were found to be appropriate for H<sub>2</sub> production via thermophilic fermentation. Previously, the highest yield of 39 mL-H<sub>2</sub>/g VS was reported at F/M ratio of 6 (Pan *et al.*, 2008).

Thus in this study, the extent of efficiency of CO<sub>2</sub> capturing method using 80% (w/v) NaOH solution was investigated. The optimum B/M ratio was also determined for an enhanced H<sub>2</sub> production via anaerobic fermentation in thermophilic reaction using cooked rice as substrate. The combined effect of carbon dioxide capture and the optimum B/M ratio was investigated since there were no studies if these two fermentation conditions can significantly affect the H<sub>2</sub> production in an anaerobic fermentation condition.

## Materials and Methods

### Preparation of Sludge

Mesophilic seed sludge for the experiment was collected from Yongin Waste Water Treatment Plant, Gyeonggi-do, South Korea. Preliminary 9-day analyses of the Yongin Sewage Sludge (YSS) exposed to thermophilic conditions was done to determine its optimum production capacity. One liter of the collected sludge was placed in 2-L flask-bioreactors and was acclimatized for at least 2 days in a thermophilic condition at 50±1°C.

After 2 days, evolved gas in the bioreactors was analyzed using Gas Chromatography (GC) to determine the activity of the sludge. Other parameters like pH, Total Solid (TS), Volatile Solid (VS), protein, carbohydrate and lipid concentration were determined according to the Standard Methods for the Examination of Water and Wastewater Treatment (Eaton *et al.*, 1995). Protein concentration was measured through Bio-Rad Assay for BSA using a Shimadzu UV-Visible Spectrophotometer UV-160 1PC at 595 nm. The carbohydrate concentration was determined in terms of glucose (CH<sub>2</sub>O) units at 490 nm (Eaton *et al.*, 1995). Results were analyzed against a standard calibration curve for protein and carbohydrate concentrations.

### Substrate Preparation

Rice as the substrate was cooked with water in a 1:1 (v/v) ratio using a rice cooker and was finely grounded using a Hanil Cooking Mixer, HMF-347(E) model. The parameters TS, VS, protein concentration, CH<sub>2</sub>O concentration and lipid concentration were determined when the grounded rice were mixed with the inoculums before and after anaerobic fermentation. The amount of substrates for the different B/Ms, was based on the theoretical ratio of 4.17 parts of cooked rice to 0.83 parts of Acclimatized Seed Sludge (ASS) for B/M of 5, 6.12 parts of cooked rice to 0.88 parts of ASS for B/M of 7 and 8.1 parts of cooked rice to 0.9 parts of ASS for B/M of 9.

### Thermophilic Anaerobic Digestion Set-Up and Operation

A series of batch experiments in duplicate, were conducted using five one-Liter glass amber bottles (SCHOTT Duran, Germany) sealed with a rubber stopper screwed with a plastic cap with sampling gas ports. Each of the three bottles was supplied with the ASS and rice with a variable ratio of B/Ms of 5, 7 and 9 with VS loading equivalent of 3.0 gVS/L and the other two bottles served as the control set-ups supplied with ASS but without rice as substrate. Carbon dioxide capturing was done by hanging a 20 mL-vial with 3.0 mL 80% (w/v) NaOH solution at the headspace of the four bioreactors attached to the rubber stopper. Anaerobic fermentation was conducted with a total working volume of 0.35 L for each bioreactor. Each bioreactor was purged with nitrogen (N<sub>2</sub>) gas for 10 min before placing it in a shaking incubator (Vision Scientific Co., LTD KMC-84080SF model) with an agitation speed of 100 rpm at 50±1°C temperature. The initial pressure and pH in each bioreactor were determined before placing them in the incubators.

### Analytical Methods

Gas samples were taken from the headspaces of the bioreactors. The amounts of biohydrogen generated from the batch fermentation were detected using GC (HP 6890 GC Method) equipped with thermal conductivity detector. Analytes were separated using packed column Cat19808 Model Number Restek with Shin Carbon ST 100/120 and N<sub>2</sub> as the carrier gas. The temperature of the injector, oven and detector were kept at 120, 150 and 180°C respectively with a total flow of 12 mL/min. The reference and make-up gas flow rates were set at 20 mL/min and 7 mL/min, respectively. A biogas standard (Scott Specialty Gases, Plumsteadville) composed of CH<sub>4</sub> (30% v/v), H<sub>2</sub> (30.1% v/v) and CO<sub>2</sub> (39.9% v/v) was used for calibration. The volume of the gas produced was determined using the pressure

generated at the headspace as measured through a pressure transducer (WAL\_BMP test system 3150. Oldenbrug) and calculated using the Ideal gas law equation corrected to standard conditions as described by Pan *et al.* (2008). After detection of the pressure, the biogas is released and the pressure in the headspace is again measured to serve as the initial pressure for the next sampling detection. The daily pressure detection served as the final pressure for the computation of the final volume produced using the ideal gas equation.

In all conditions for the batch experiments, the CHP was fitted to a modified Gompertz Equation (Equation 1) used as a suitable model for describing the H<sub>2</sub> production in batches (Zhang *et al.*, 2003; Chen *et al.*, 2006; Pan *et al.*, 2008; Zhang *et al.*, 2007; Sreela-or *et al.*, 2011):

$$H = P \exp \left[ -\exp \left\{ \frac{R_m e}{P} (\lambda - t) + 1 \right\} \right] \quad (1)$$

In Equation 1, *H* represents the cumulative volume (mL) of H<sub>2</sub> produced at time *t* (h), *P* the H<sub>2</sub> production potential (mL), *R<sub>m</sub>* the maximum H<sub>2</sub> production rate (mL/hr), *λ* the lag phase time (h) and *t* the incubation time (*h*) (Sreela-or *et al.*, 2011). Pearson product moment correlation coefficient (*r*<sup>2</sup>) for different trials in each sampling was used to establish reliability of the results. One-Way ANOVA for 5 independent samples and pair-wise comparisons via Tukey HSD Test were used as a statistical treatment to determine the significance of the results.

## Results and Discussion

The preliminary profile for at least 9-day analyses of the sludge under thermophilic condition was used to determine the number of days required for acclimation to optimized H<sub>2</sub> production before fermentation conditions were enhanced. Results showed that growth activity phase of the YSS starts after a day of acclimation and the optimum potential for hydrogen production is at least 2 days. These results were used as the bases for a 2-day acclimatization of the seed sludge for anaerobic batch fermentation. Such pretreatment conditions were used for anaerobic fermentation to optimized H<sub>2</sub> production in all series of batch experimentation.

### Substrate Degradation

The ASS was used for all the bioreactors at different B/Ms with rice as the substrate. The two bioreactors that served as the control both had only ASS without the substrates but one control set-up was with CO<sub>2</sub> capture method and the other, was without the CO<sub>2</sub> capture method. Before and after the anaerobic fermentation, physical and chemical characteristics of the solution were examined and Table 1 shows the comparison.

Table 1. Physical and chemical Parameters of the bioreactors under study

Parameters	B/M 5	B/M 7	B/M 9	Control 1*	Control 2**
Initial pH	7.86	7.73	7.66	7.81	7.80
Final pH	4.33	4.08	3.73	9.00	7.89
VS reduction (mg/L)	800.00	650.00	1600.00	1000.00	1150.00
Carbohydrate conversion (mg/L)	10.00	8.00	16.00	9.00	23.00
Protein (mg/L)	71.00	76.00	37.00	85.00	82.00
Lipid (mg/L)	875.00	1750.00	900.00	1100.00	750.00

The values of the different parameters in Table 1 indicated an enhancement of H<sub>2</sub> production in an anaerobic fermentation. The values in mg/L for VS reduction, carbohydrate conversion, protein and lipid contents, during anaerobic fermentation provide evidence of the extent of substrate degradation for the production of H<sub>2</sub>. The final pH of 4.0±0.3 (initial pH = 7.8±0.1) at all B/Ms validated the H<sub>2</sub> production as pointed out by some studies on the VFA changes before and after fermentation reactions (Zhang *et al.*, 2003; Hao *et al.*, 2006). Fermentations at all B/Ms were remarkable as compared to the two control set-ups with final pH of 9.00 and 7.89. The reduction of VS is greatest at B/M of 9 with 1600 mg L<sup>-1</sup> wherein it also had a higher carbohydrate conversion. Apparently, the hydrogen-producing bacteria in the bioreactor with B/M 9 readily converted the soluble part of rice into hydrogen as compared to B/M7 and B/M 5. Nevertheless, fermentation at all B/Ms manifested VS reduction and carbohydrate conversion for the bioreactors under study. It can be deduced from the values that the hydrogen-producing bacteria had utilized the carbohydrates for their growth and organic acid production as manifested by its protein yield and lipid-increase. Protein yield is highest at B/M of 7 with an evidence of having the greatest lipid profile. The sludge is an organic industrial waste and may contain high amounts of lipids (Angelidaki and Ahring, 1992). Aside from the degradation of the hydrolysate, the response of a biogas process to addition of neutral lipids that is part of the inoculums may depend upon the degree of adaptation. During degradation in an anaerobic fermentation, hydrolysis of carbohydrates and other nutrients to glycerol and long fatty acids proceeds very fast and is further degraded to acetate and hydrogen. The hydrogen produced may then be further consumed if acetogenesis or hydrogenotrophic methanogenesis is not inhibited.

#### Effects of Carbon Dioxide Capture on Hydrogen Production

Anaerobic fermentation reactions are accompanied by CO<sub>2</sub> and HCO<sub>3</sub><sup>-</sup> formation (Meier-Scheiders *et al.*, 1995). CO<sub>2</sub> is believed to be released from the cells into the solution in its dissolved form. Depending on the pH and partial pressure of CO<sub>2</sub> in the fermentation, significant amounts of liquid CO<sub>2</sub> can be in equilibrium with the gaseous CO<sub>2</sub> or may formed into acetates. Fermentation in

this study was run between pH 7.1±0.1 and 4.0±0.3, so significant amounts of CO<sub>2</sub> in the gaseous phase can be released. As soon as the gaseous CO<sub>2</sub> concentration is above the partial pressure in the headspace, desorption took place inside the vial containing the NaOH (80% w/v) favoring H<sub>2</sub> production along the process.

Figure 1 shows the cumulative biogas production (A) and H<sub>2</sub> production (B) at varying B/Ms with CO<sub>2</sub> capture in the fermentation reactions. The bioreactor with B/M of 9 exhibited the highest Cumulative Hydrogen Production (CHP) of 138.40 mL which is 41.58% of the total biogas production as compared to 95.23 mL (33.81%) at B/M of 5 and 88.95 mL (32.80%) at B/M of 7. Since there were no CH<sub>4</sub> and CO<sub>2</sub> gases present in the bioreactors with B/M 5, 7 and 9, the remaining biogas is believed to be N<sub>2</sub> and were not detected since the carrier gas of the GC was N<sub>2</sub> gas.

The highest average H<sub>2</sub> production of 51.83 mL-H<sub>2</sub> was attained after 49.1 h at B/M of 9. B/M 7 had an average of 39.97 mL-H<sub>2</sub> after 86.86 h and B/M 5 had 32.19 mL-H<sub>2</sub>. From the results, it can be noted that the presence of CO<sub>2</sub>-capture system inside the bioreactors favored H<sub>2</sub> production for all B/Ms tested. The presence of H<sub>2</sub> in the biogas production for all bioreactors with the CO<sub>2</sub> capturing method pointed to the enhancing capacity of 80% (w/v) NaOH solution inside the bioreactors especially at B/M of 9.

#### Comparison of Hydrogen Yield with other Biogas

Comparison of the other biogas yield during fermentation reaction at different B/Ms and the two control set-ups is shown in Fig. 2.

In the Figure, no CH<sub>4</sub> and CO<sub>2</sub> gases were generated and only H<sub>2</sub> was produced in all the varying B/M ratios with B/M of 9 exhibiting the highest H<sub>2</sub> yield. On the other hand, negligible H<sub>2</sub> was produced in both control set-ups with control 2 having slightly higher CO<sub>2</sub> levels due to the absence of CO<sub>2</sub> capture system. The absence of CH<sub>4</sub> and CO<sub>2</sub> as by-products of the anaerobic fermentation at all B/Ms can be due to a number of reasons. First, the acclimatization of YSS at 50°C removed the non-spore-forming methanogens from the inoculums (Zhang *et al.*, 2003; Kim *et al.*, 2004) though some results in another study showed that heat-pretreatment inoculums did not enhance hydrogen yield (Luo *et al.*, 2010). Second, the thermophilic condition during the entire fermentation reaction

prevented the microbial formation of the interspecies hydrogen to methanogens, thus recovering the H<sub>2</sub> gas in the process (Zhang *et al.*, 2003). An increase of 69.6 mLH<sub>2</sub>/gVS under thermophilic conditions was observed in some studies (Luo *et al.*, 2010) and there was a yield of 56.6 mLH<sub>2</sub>/gVS and 249 mLCH<sub>4</sub>/gVS under high organic loading rate (>10 gVS/(L.d) from a two-phase thermophilic CSTR for hydrogen and methane production. The temperature proved to be more important and hydrogen production can be maintained at a more stable condition of 55°C without

significant hydrogen consumption (Lin *et al.*, 2012). Third, the reduction of pH during fermentation from pH 7.8±0.1 to pH 4.0±0.3 and the constant temperature of 50±1°C consistently eliminated the production of measurable concentration of methane and produced greater H<sub>2</sub> yields confirming previous studies (Oh *et al.*, 2003; Nazlina *et al.*, 2009). Lastly, the dominating presence of H<sub>2</sub> in the three bioreactors (B/Ms of 5, 7 and 9) favors CO<sub>2</sub> capture and prevented acetogenesis and hydrogenotrophic methanogenesis during the entire fermentation.

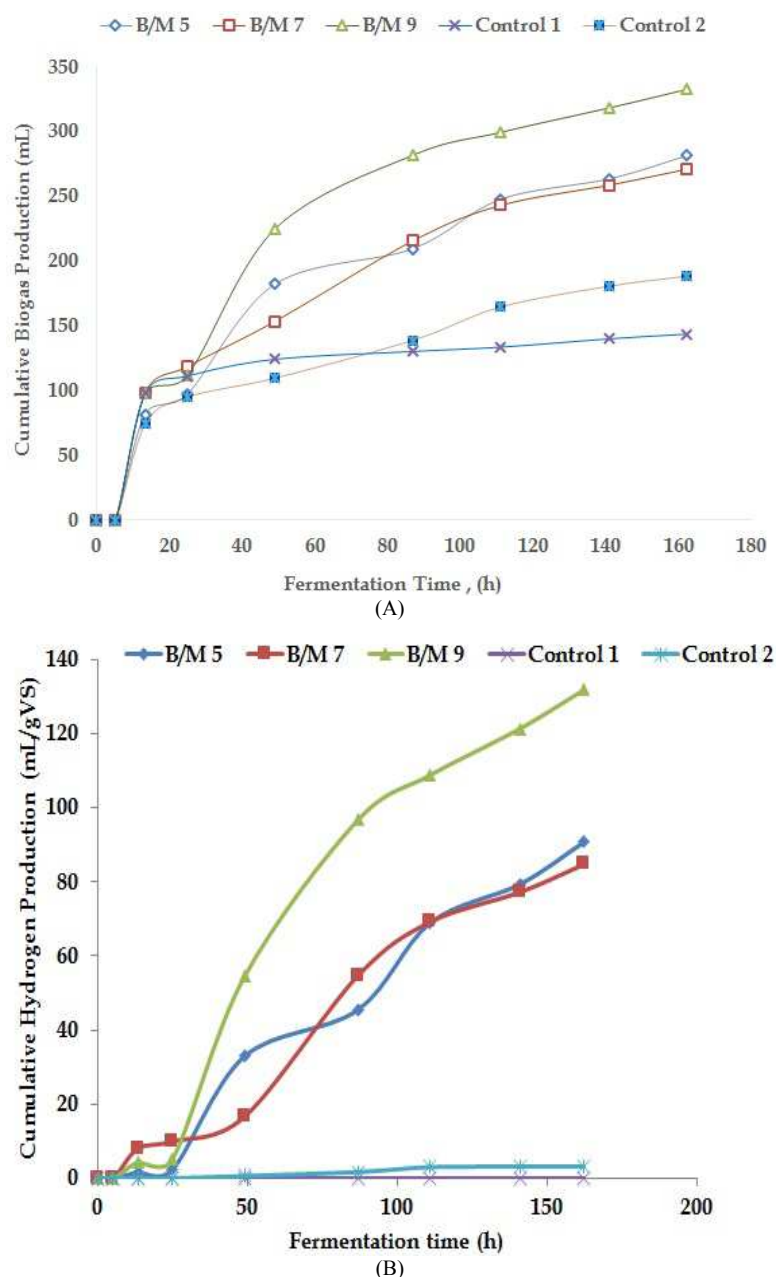


Fig. 1. Cumulative biogas production (A) and cumulative hydrogen (H<sub>2</sub>) production (B) at varying mass to microorganism ratio during fermentation with carbon dioxide (CO<sub>2</sub>) capture

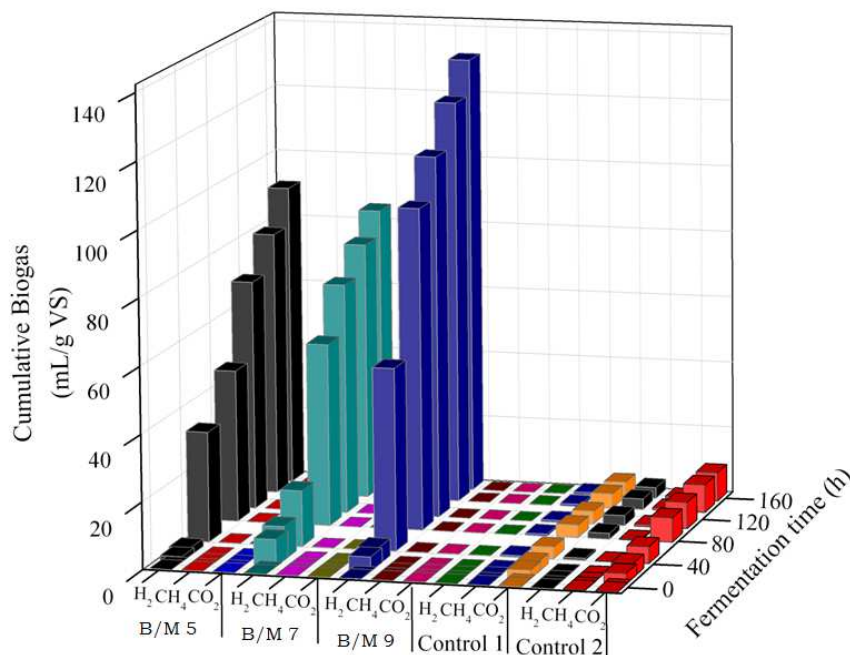


Fig. 2. Comparing biogas production; methane (CH<sub>4</sub>) and carbon dioxide (CO<sub>2</sub>) with hydrogen (H<sub>2</sub>) production at varying B/M ratio with the use of CO<sub>2</sub> capture in an anaerobic fermentation

Table 2. Kinetic Parameters and hydrogen production yield at different B/M ratio and time

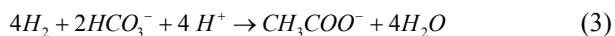
B/M ratio	Lag phase RS: SS λ (h)	Hydrogen production rate, R <sub>m</sub> (mL/h)	Hydrogen potential P (mL/gVS)	r <sup>2</sup>	Hydrogen production H (mL)	
					after 5 h	after 10 h
B/M 5	4.21	1.27	90.70	0.92	6.49	10.25
B/M 7	4.79	1.02	84.72	0.97	5.7	8.55
B/M 9	5.46	4.27	131.81	1.00	7.77	21.29
Control 1*	49.9	4.61×10 <sup>-5</sup>	0.06	0.83	4.45×10 <sup>-5</sup>	9.28×10 <sup>-5</sup>
Control 2**	4.52	2.68×10 <sup>-2</sup>	3.27	0.91	3	4

\*with CO<sub>2</sub> capture; \*\* w/o CO<sub>2</sub> capture

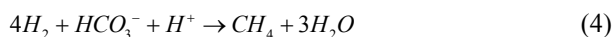
The fermentation reaction can be generalized in the following reaction:



At such mechanism of the fermentation reaction, two possible chemical reactions may take place; one is acetogenesis from CO<sub>2</sub> and H<sub>2</sub>:

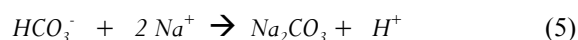


Another possibility is the hydrogenotrophic Methanogenesis:



Such two possible reactions consume hydrogen in the process to produce methane or acids. With the presence

of the hanging vial containing 3.0 mL of 80% (w/v) NaOH solution inside the bioreactors, the following reaction have taken place in the atmosphere as CO<sub>2</sub> capture; thus producing a hydrogen-rich region in space:



In this study, the highest average H<sub>2</sub> yield of 51.83 mL-H<sub>2</sub> after 49.1 h at B/M of 9 with an initial 3.0 gVS/L and without methane production can be attributed to the presence of carbon dioxide capture in the bioreactors.

#### Kinetic Analysis

In terms of the kinetic parameters, comparison of the H<sub>2</sub> production across the varying B/M ratio using Gompertz equation is shown in Table 2. A theoretical value using Equation 1 computed at 5 and 10 h were

compared based on the kinetic parameters of the different B/Ms.

The values of the lag phase for all the 3 B/M ratio were not significantly different except the control 1 set-up but the values of the production rate provided a significant difference in terms of the hydrogen potential and the hydrogen production. In all the bioreactors, B/M of 9 has the highest production rate of 4.27 mL h<sup>-1</sup>, thus producing the highest hydrogen production as compared to the other B/M ratio of the substrate to ASS. The longer lag phase time for control 1 set-up can be due to the absence of substrate and the presence of carbon dioxide capture. The hydrogen producing bacteria of the inoculums must have starved to death and had longer time for their adaptation to the environment to produced negligible volume of hydrogen and carbon dioxide as compared to control 2 set-ups (without CO<sub>2</sub> capture) producing slightly higher volume of H<sub>2</sub> and greater volume of CO<sub>2</sub>. The Pearson product moment correlation, r<sup>2</sup>, for the duplicates in all samples for each food to microorganism ratio with the two control set-ups provided a valid correlation for B/M 9 and the other two B/Ms.

#### Statistical Analysis

To determine the significance of the results, one-way ANOVA for 5 independent samples were used to treat the values of CHP across the 3 B/Ms and the two control set-ups. Table 3 shows the summary of the One-way ANOVA of the five bioreactors.

The F-value of 4.79 at P α 0.003 points a significant difference among the values across the different B/M ratio as compared to the two control set-ups. Since there were 5 independent groups compared, further analysis using Pair-wise Comparisons via Tukey HSD test was done to further identify which B/M ratio is significantly different from the other B/M ratios and the two control set-ups. Table 4 further pointed out the significance of the result.

The CHP values of the bioreactor with B/M 5 showed no significant result as compared to B/M of 7 and 9 and the two control set-ups during anaerobic fermentation reaction. Results also showed that B/M of 7 did not significantly differ from B/M of 9 and the two control setups. Although B/M of 9 did not significantly differ from B/M of 5 and 7 but it is significantly different as compared to the two control set-ups. The result showed that there was a significant difference in the CHP of the bioreactors at B/M of 9 with the use of CO<sub>2</sub> capture as compared to the two control set-ups of not having the CO<sub>2</sub> capture.

#### Findings

The CO<sub>2</sub> capture method using the 80% (w/v) NaOH solution enhanced H<sub>2</sub> production for all bioreactors especially at B/M of 9 with the highest hydrogen yield of 55.85 mL H<sub>2</sub>/gVS. This was better than the highest yield of 39 mL-H<sub>2</sub>/g VS as reported for F/M ratio of 6 (Pan *et al.*, 2008). The result further identify that there was not much significance across the variable B/M ratio as predictor for maximum H<sub>2</sub> production. The significant difference was brought about by the CO<sub>2</sub> capture method in the bioreactor containing B/M ratio 9 which was not present in the two control set-ups confirming other findings that the method favors H<sub>2</sub> production.

The results in these data confirmed the findings of previous researches (Park *et al.*, 2005; Zhang *et al.*, 2007; Hao *et al.*, 2006) that H<sub>2</sub> production can be enhanced with thermophilic condition maintained all throughout the fermentation. Moreover, the loss of hydrogen through interspecies to promote methane can be prevented by agitation, pH and temperature control since the batch fermentation started out with pH 7.8±0.1 in all conditions and the shaking condition was 100 rpm, incubated at thermophilic conditions. The combination of shaking with the CO<sub>2</sub> capture at the headspaces of the bioreactors produced a better condition for a H<sub>2</sub> production especially at B/M of 9.

Table 3. Means and F-ratio from One-way ANOVA of 5 bioreactors

Bioreactors	Mean CHP	Standard deviation	F-ratio	Probability level
B/M 5	35.69	37.03	4.79	0.003*
B/M 7	35.60	35.27		
B/M 9	58.06	56.98		
Control 1	0.01	0.02		
Control 2	1.33	1.48		

\*significant at 0.01 level

Table 4. Pair-wise comparison via Tukey HSD test

Bioreactors	B/M 7	B/M 9	Control 1	Control 2
B/M 5	n/s*	n/s	n/s	n/s
B/M 7		n/s	n/s	n/s
B/M 9			p<0.01	p<0.01
Control 1				n/s

The increase in the H<sub>2</sub> production must result from an aspect related to the reduced CO<sub>2</sub> concentration in the system. The most likely explanation is that a reduction in CO<sub>2</sub> concentration reduced hydrogen losses via acetogenesis which could not be completely inhibited<sup>17</sup> and the shaking condition of the bioreactor. The results clearly affirmed the findings of the previous researches that CO<sub>2</sub> trapping or scavenging is an important condition for better results (Stolaroff *et al.*, 2008; Figueroa *et al.*, 2008; Lewis *et al.*, 2011). Likewise the shaking conditions or stirring also enhances the production of hydrogen especially when the anaerobic conditions are thermophilic. Furthermore, the B/M ratio of 9 with continuous CO<sub>2</sub> capture method using 80% (w/v) NaOH solution provided an enriched H<sub>2</sub> region in the atmosphere, which enhanced its generation.

## Conclusion

The result of this investigation concluded that capturing CO<sub>2</sub>, with 80%(w/v) NaOH solution in the atmosphere of bioreactors in an anaerobic fermentation favors an enhanced production of H<sub>2</sub> and providing a mechanism for H<sub>2</sub>-rich atmosphere thus preventing further hydrogenotrophic methanogenesis and acetogenesis in the reaction especially for Biomass to Microorganism (B/M) ratio of 9.

The anaerobic fermentation in batches with the carbon dioxide (CO<sub>2</sub>) scheme capture proved to be an effective means of redirecting the methanogenesis pathway to the production of more volume of hydrogen in the process. This condition of capturing carbon dioxide should be considered when doing anaerobic fermentation for an enhanced hydrogen (H<sub>2</sub>) production. The present study proved that utilizing sodium hydroxide as a compatible reagent for carbon dioxide capture for an enhanced hydrogen production and redirecting the pathway of mechanism to control the production of methane, provide better hydrogen yield. Similar studies using reagents with similar reactivity for better hydrogen yield may be investigated in the near future with considerations on the concentrations of the substance used for carbon dioxide capture.

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## Author's Contributions

**Alma Negre Abug:** Conceived the design and performed the batch experiments and conducted statistical analyses and wrote the manuscript.

**Young Sook Oh:** Contributed to the conceptual development, design and review the manuscript for significant intellectual content.

## Ethics

The study was conducted without the involvement of human and animal subjects and there is no conflict of interest whatsoever between the authors.

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