Repeated-batch Fermentative for Bio-hydrogen Production from Cassava Starch Manufacturing Wastewater

Suksaman Sangyoka, Alissara Reungsang and Samart Moonmart
Rachjababt Phiboonsongkram University, Phitsanuloke, 65000 Thailand
Department of Biotechnology and Fermentation Research Center for Value Added Agricultural Products, Faculty of Technology, Khon Kaen University, A. Muang, Khon Kaen, 40002, Thailand

Abstract: Anaerobic hydrogen production from cassava wastewater by heat-treated UASB granules was conducted in a 10 L bioreactor with a working volume of 8 L at room temperature and pH 6.0 by batch and repeated-batch fermentations. Specific hydrogen production potential, hydrogen yield and the maximum hydrogen production rate of 39, 304.81 mL, 0.22 mL mg-COD⁻¹ and 851.84 mL h⁻¹, respectively, were obtained in a batch reactor. A repeated-batch was conducted when the glucose concentration in fermentative broth was depleted to 150-250 mg L⁻¹ which equivalent to 10-15% of initial glucose concentration. Repeated-batch reactor was operated at 3 different feed-in/feed-out rates i.e., 25, 50 and 75%. Results revealed that a suitable feed-in/ feed-out rate for production of hydrogen from cassava wastewater was at 75%. This was indicated by the highest hydrogen yield, the highest potential maximal amount of hydrogen produced, a relatively high maximum hydrogen production rate, a relatively high maximum specific hydrogen production rate and a relatively short lag time of 0.0094 mL mg-COD⁻¹, 12.532.80 mL, 540.46 mL h⁻¹, 3.5 mL g-VSS⁻¹ h⁻¹ and 5.31 h, respectively. Major soluble metabolites were acetate and butyric acids. Our results indicated that repeated batch fermentation was more effective in producing hydrogen from cassava wastewater than batch fermentation.

Key words: Bio-hydrogen, cassava wastewater, repeated-batch fermentative, UASB granules

INTRODUCTION

Hydrogen is considered to be an ideal and clean source of energy for the future because no carbon dioxide is produced and water is the only product as a result of its combustion. Nowadays, there are many processes involving in hydrogen production such as gasification of coal (Winter and Nitsch, 1988), stream reforming of natural gas (Williams et al., 1995), electrolysis of water (Kato et al., 2005), thermal decomposition of water (Krusel et al., 2002) and biological hydrogen production (Fumiki et al., 1996; Yokoi et al., 1997). Among these processes, microbial hydrogen production is an attractive process because there are many fermentative bacteria capable of producing hydrogen with a low capital cost according to extended periods of viable cells used. Various kinds of organic substrates could be used for producing hydrogen such as glucose (Mu et al., 2006), sucrose (Tao et al., 2007; Hussey et al., 2005) and agricultural products such as sugar beet (Hussey et al., 2005). Moreover, organic waste such as sugary wastewater (Ueno et al., 1996), esterification wastewater (Huang et al., 2004) and starch-manufacturing waste (Yokoi et al., 2002) had been reported to be used as substrates for hydrogen production with the advantages of reducing cost of substrate for hydrogen fermentation and also disposal cost of wastes.

Thailand is the world’s largest exporter of cassava, contributes to about 80% of total world exports (Food and Agriculture Organization of United Nations, 2000). Starch is recovered from cassava roots by wet processing which generates large volume of high strength liquid stream that is considered as wastewater. One kilogram of fresh cassava roots yields about 0.2 kg of starch, 0.4-9 kg of cake and about 5-7 L of wastewater in the starch production (Richard and David, 2004). The wastewater has a very high Chemical Oxygen Demand (COD), Biochemical Oxygen Demand (BOD) and total solid. Thus disposal of high strength cassava starch manufacturing wastewater poses a burden on the environment. Cassava wastewater contains Volatile Fatty Acid (VFA) which are the intermediates for producing hydrogen i.e., acetate, propionic and butyric acids of approximately 415.78, 565.95 and 863.50 mg L⁻¹,
respectively (Polprasert and Rukvichitkul, 2003). In these instances, cassava wastewater can be a good candidate for bio-hydrogen production.

Biological hydrogen production could be conducted in either batch (Yokoi et al., 1997), repeated batch (Yokoi et al., 2002; Chin et al., 2003) or continuous fermentation processes (Chang and Line, 2004). Repeated batch fermentation is an attractive technique because it is the noncomplex technique in which hydrogen could be continuously produced with the decreasing in lag time and increasing in total volume of hydrogen produced (Yokoi et al., 2002). There were few reports on hydrogen production in repeated batch fermentation which could provide more effective hydrogen production than in batch fermentation (Yokoi et al., 2002; Vazquez et al., 2005). To our best knowledge, there was no report on hydrogen production from cassava wastewater by repeated-batch fermentation process.

Therefore, this research was conducted to investigate a repeated-batch fermentative for bio-hydrogen production from cassava wastewater using heat-treated anaerobic sludge granules as the inoculum. The feed in/ feed out rates for repeated batch experiment were varied to be 25, 50 and 75% in order to determine the optimum feed in/ feed out rate for successive hydrogen production from cassava wastewater. Information from this study would provide a promise renewable energy technology while simultaneously solving waste disposal problems in the cassava starch industry.

**MATERIALS AND METHODS**

**Inoculum:** Anaerobic granular sludge used in this study was obtained from the upflow anaerobic sludge blanket reactor of Khon Kaen Brewery Co. Ltd. in Khon Kaen Province, Thailand. The granules had pH, VSS and TSS of 6.87, 25,430 and 19,300 mg L⁻¹, respectively. Granules were heat-treated at 100°C in water bath for 30 min in order to inhibit methane producing microorganisms prior the usage as inocula for hydrogen production.

**Cassava wastewater:** Cassava wastewater used as substrate for hydrogen production in this study was obtained from Asia Modify Starch Factory, Kalasin Province, Thailand. The compositions of the wastewater were shown in Table 1. The final COD:N:P ratio of wastewater was adjusted to be 100:10:1 by supplementing with NH₄Cl and K₂HPO₄ as N and P-sources, respectively, before using as substrate for hydrogen production.

**Reactor and experimental procedure:** The hydrogen production from cassava wastewater was conducted in 10 L bioreactor with a working volume of 8 L at room temperature, pH 6 in batch in comparison to repeated batch experiment. The schematic of the reactor was illustrated in Fig. 1. The batch experiment was conducted by adding the heat-treated granules at the concentration of 10,000 mg L⁻¹ into cassava wastewater in the bioreactor. The bioreactor was then purged with argon for at least 15 min to ensure anaerobic condition before starting the fermentation process with agitation rate of 50 rpm to prevent the settling down of the granules to bottom of the reactor. The hydrogen evolution and glucose concentration and volatile fatty acids (VFAs) i.e., acetic, butyric and propionic acids in fermented broth were determined every 6 h until hydrogen evolution and glucose concentration was observed to be stable.

The repeated batch reactor was operated by varying the feed-in/ feed-out rates to be 25, 50 and 75% and hydrogen production and glucose concentration were monitored throughout the experiment. At each feed-in/ feed-out rate, a fresh substrate was used to replace the culture broth in the bioreactor when cumulative hydrogen production was stable (as an observed value from batch experiment). The hydrogen production was investigated every 6 h for 250 h.

**Analytical method**

**Gas analysis:** The volume of biogas produced was measured by gas meter and the analysis of biogas composition was performed using a gas chromatography (GC) (Model 2021, SHIMADZU, Japan) equipped with a thermal conductivity detector (TCD). A 2 m × 2.5 mm stainless-steel column packed with 60/80 mesh Unibead C was used to analyze the percentage of hydrogen, nitrogen, methane and carbon dioxide in the biogas produced. Helium was used as the carrier gas at a flow rate of 25 mL min⁻¹. The temperature of injector, detector and column were 150, 150 and 145°C, respectively.

<table>
<thead>
<tr>
<th>COD</th>
<th>BOD</th>
<th>TN (mg L⁻¹)</th>
<th>TP</th>
<th>TSS</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>22,600</td>
<td>12,600</td>
<td>258</td>
<td>54</td>
<td>1,198</td>
<td>5.02</td>
</tr>
</tbody>
</table>

**Table 1: Wastewater characteristics of Asia Modify Starch Factory, Kalasin Province**
Volatile fatty acids (VFAs): Concentrations of acetic, propionic and butyric acids were determined by a gas chromatography (Model 14B, SHIMADZU, Japan) equipped with a flame ionization detector (FID) and a 3 m × 3.2 mm glass column packed with 30/60 mesh Carbowax 20M. Injector, detector and column temperatures were at 200, 250 and 180°C, respectively. Nitrogen, hydrogen and pressurized air were used as carrier gases with flow rate of 80, 70 and 500 mL min⁻¹, respectively.

Kinetic analysis: The cumulative hydrogen production in the batch experiment was analyzed following the modified Gompertz equation (Lay et al., 2001)

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

Where \( H \) represents the cumulative volume of hydrogen produced (mL), \( P \) is the hydrogen production potential (mL), \( R_m \) is the maximum production rate (mL h⁻¹) and \( \lambda \) is the lag time (h). The maximum specific hydrogen production rate (mL g-VSS⁻¹ h⁻¹) was calculated by dividing \( R_m \) by the initial sludge VSS. The hydrogen yield (mL g-COD⁻¹) was calculated by dividing \( P \) by the quantity of COD of cassava wastewater.

RESULTS AND DISCUSSION

Batch hydrogen experiment: The hydrogen evolution from cassava wastewater by heat-treatment anaerobic granular sludge was conducted in batch experiment. Concentration of glucose in fermentative broth and hydrogen production were monitored over time. Results indicated that glucose concentration was depleted during the fermentation process until 72 h (Fig. 2a) indicating the use of glucose in cassava wastewater as energy source for hydrogen production. After 72 h, glucose concentration in fermentative broth was found to be stable at the concentration of 150-250 mg L⁻¹ (10-15% of initial glucose concentration) (Fig. 2a). This result coincided with the amount of hydrogen produced in which the cumulative hydrogen was observed to be stable at the volume of approximately 18 L after 72 h of fermentation (Fig. 2b) suggesting a lack of energy source.

Fig. 2: Culture performance of the anaerobic batch cultivation of granule for hydrogen production at ambient and pH 6.0. Time profiles of (a) glucose consumption, (b) cumulative hydrogen, (c) volatile fatty acid (d) HBu/HAc ratio
for producing hydrogen though the cells can still be survive with a little amount of glucose left in the substrate (150-250 mg L\(^{-1}\)).

The major products observed during the fermentation process were acetic, butyric and propionic acids (Fig. 2c). Results revealed that there was a correlation between hydrogen production and produced VFA. After 66 h, an accumulated hydrogen starting to reach a stationary phase while butyric acid increased.

A ratio of HBu/HAc was calculated to evaluate hydrogen production. Results indicated that after 66 h, HBu/HAc increased (Fig. 2d). An increase of HBu/HAc ratios indicated a limitation of butyric acid transforming to acetic acid resulting in a low amount of hydrogen produced as described in following equation (Fung et al., 2005).

\[
\text{CH}_3\text{CH}_2\text{CH}_2\text{COOH} + 2\text{H}_2\text{O} \rightarrow 2\text{CH}_3\text{COOH} + \text{H}_2 \quad \text{butyric} \quad \text{acetic} \quad (2)
\]

A decrease in glucose concentration (Fig. 2a) which led to a decrease in hydrogen produced (Fig. 2b) suggested that the fresh substrate should be added immediately after the glucose concentration in culture broth was depleted to 150-250 mg L\(^{-1}\) (10-15% of initial glucose concentration) in order to supply energy source and to dilute the cumulative VFA which might inhibit hydrogen production in our process.

The modified Gompertz equation was used to describe the cumulative hydrogen production process obtained from a batch experiment. The correlation coefficient values of 0.99 indicated a good fit of the data to the modified Gompertz equation. The observed values of the potential maximal amount of hydrogen production, the maximum hydrogen production rate and the lag time in batch experiment were 39,304.81 mL, 851.84 mL h\(^{-1}\) and 27.16 h, respectively. Results showed that high hydrogen yield of 0.22 mL mg-COD\(^{-1}\) was produced with the maximum specific hydrogen production rate of 5.54 mL g-VSS\(^{-1}\) h\(^{-1}\) indicating that cassava wastewater could be effectively used as a substrate for hydrogen production by heat-treated anaerobic sludge granule in the batch experiment.

**Repeated-batch hydrogen production:** The repeated batch culture was conducted based upon the data from batch culture in which the culture broth in the reactor was replaced by a fresh substrate when glucose concentration in broth was decreased to 150-250 mg L\(^{-1}\) which was equivalent to 10-15% of initial glucose concentration in cassava wastewater. The repeated batch experiment was conducted with varied feed-in/feed-out rates of 25, 50 and 75%. The hydrogen production and glucose consumption profiles in repeated batch culture were shown in Fig. 3. Results revealed that in each batch, glucose was consumed by heat-treated anaerobic sludge to produce hydrogen from cassava wastewater as shown in (Fig. 3a, b and c). Hydrogen evolution was observed to be stable after eighth, seventh and forth batch culture for 25% (Fig. 3d), 50% (Fig. 3e) and 75% (Fig. 3f) feed in/feed out rates, respectively. The average feeding time for the feed in/feed out rate of 25, 50 and 75% were 12, 21 and 36 h, respectively (Fig. 3).

At each batch, a modified Gompertz equation was used to describe the cumulative hydrogen production process. The modified Gompertz equation parameters were shown in Table 2. The correlation coefficient values of 0.99 indicated a good fit of the data to the modified Gompertz equation. Results suggested that a suitable feed-in/feed out rate for production of hydrogen from cassava wastewater was at 75% indicated by the highest potential maximal amount of hydrogen production, a relatively high maximum hydrogen production rate, a relatively high specific hydrogen production potential and the highest hydrogen yield of 12,532.80 mL, 540.46 mL h\(^{-1}\), 3.50 mL g-VSS\(^{-1}\) h\(^{-1}\) and 0.0094 mL mg-COD\(^{-1}\), respectively, though the longer lag time was observed at this feed in/feed out rate (Table 2). It was important to note that the maximum specific hydrogen production rate at 75% feed in/feed out rate was lower than that at 50% (20.81%).

In comparison of repeated batch to batch experiment, results indicated that repeated batch was more effective than the batch culture in which lag time to the exponential phase was reduced from 27.16 (batch) to 1.02 h (25% feed in/feed out), 2.41 h (50% feed in/feed out) and 5.31 h (75% feed in/feed out), respectively. This might be
due to the fact that in repeated-batch the substrate (cassava wastewater) was periodically supplied during the fermentation resulting in continuously hydrogen production without the shortage of substrate.

Total hydrogen production in repeated batch fermentation was calculated by summing all volume of hydrogen produced from each batch and shown in Fig. 4. Results indicated that total volume of hydrogen produced in repeated-batch fermentation was higher than in batch culture at the same time of incubation, which suggested the advantage of repeated-batch over batch fermentation.

Our results were similar to the finding of Yokoi et al. (2002) who reported that total volume of hydrogen fermented from sweet potato starch waste by mixed culture of C. butyricum and Enterobacter aerogenes in repeated-batch culture was higher than batch culture. Hydrogen was continuously produced by repeated-batch fermentation with the volume of 171 mL per batch and the hydrogen yield was 2.7 mol H₂ mol-glucose⁻¹ (Yokoi et al., 2002) while the volume of hydrogen produced and the hydrogen yield obtained from batch were 105 mL and 1.7 mol H₂ mol-glucose⁻¹, respectively. The
advantage of repeated batch culture was also presented in the study of Chin et al. (2003) who conducted the hydrogen fermentation by *C. acetobutylicum* in Clostridium Growth Medium (CGM). They reported that high amount of hydrogen produced by repeated-batch was 15 folds that of the culture volume within hours. An overall hydrogen production per batch in repeated batch (Table 2) was observed to be lower than in the batch culture (39,304.81 mL). This might be resulted from the lower amount of substrate fed into the repeated batch bioreactor. However, a higher hydrogen yield was found in the batch culture (0.22 mL mg-COD⁻¹) than in the
repeated-batch experiment (Table 2). Reasons to explain this incident is still unclear.

Major VFA produced in a repeated-batch experiment were acetic, butyric and propionic acids (Fig. 5a-e). Acetic acid was observed as the dominant species throughout the repeated batch experiment at every feed in/feed out rate while butyric and propionic acids were produced in much lower amount (Fig. 5). In general, production of acetic and butyric acids favors the production of hydrogen. The maximum efficiency (yield) of hydrogen production in acetic acid fermentations is 4 mole H₂ mol-glucose⁻¹ (Ueno et al., 1996). Present results suggested that high concentration of acetic acid in the fermentation broth was an indicator of hydrogen which will be produced.

CONCLUSIONS

Cassava wastewater could be effectively used as substrate for hydrogen production by heat treated UASB granule by batch and repeated batch fermentation in which the high yield of 0.22 mL mg-COD⁻¹ was found in batch culture and 0.0042, 0.0080 and 0.0094 mL mg-COD⁻¹ were found in repeated batch with feed in/feed out rates of 25, 50 and 75%, respectively.

Feed in/feed out rate of 75% was the most suitable rate for hydrogen production from cassava wastewater by repeated-batch fermentation indicated by the highest hydrogen yield, the highest potential maximal amount of hydrogen produced, a relatively high maximum hydrogen production rate, a relatively high maximum specific hydrogen production rate and a relatively short lag time of 0.0094 mL g-VSS⁻¹ h⁻¹, 12.532.80 mL, 540.46 mL h⁻¹, 3.5 mL g-VSS⁻¹ h⁻¹ and 5.31 h, respectively. However, it was important to note that the maximum specific hydrogen production rate at 75% feed in/feed out rate was 20.81% lower than that at 50%.

A reduction in lag time, an increase in total amount of hydrogen and a capability to continuously produce hydrogen were the advantages of repeated batch fermentation over batch culture for hydrogen production.

ACKNOWLEDGMENTS

This research is supported by the Thailand Research Fund (Grant # MRG4680176) and the Ministry of Education, Thailand.

REFERENCES


