Effect of protein on biohydrogen production from starch of food waste

H. B. Ding, X. Y. Liu, O. Stabnikova and J.-Y. Wang

ABSTRACT

This study demonstrated the influence of protein on biohydrogen production from carbohydrates, especially starch, by using different combinations of two model food wastes, rice as starch-rich and soybean residue as protein-rich food waste. It was found the maximum specific hydrogen production potential, 0.99 mol H₂/mol initial starch as glucose, and the maximum specific hydrogen production rate, 530 ml H₂/h g-VS, occurred at a starch/protein ratio of 1.7. The protein content in the initial food waste not only provided buffering capacity to neutralize the volatile fatty acids as concurrent products but also enhanced the hydrogen production by providing readily available organic nitrogen such as soluble proteins and amino acids to microorganisms.

Key words | biohydrogen production, food waste, protein, solid waste, starch

INTRODUCTION

Attention has been paid most to carbohydrate rich industrial or domestic wastewaters for biohydrogen production. In the laboratory studies, simple carbohydrates such as glucose and sucrose were used as carbohydrate substrates in media to simulate the wastewaters. Some media used in previous studies on biohydrogen fermentation contained protein as the nitrogen source to enhance the growth and activity of hydrogen-producing bacteria, despite carbohydrate being universally known as the most suitable organic substrate for biohydrogen production (Bai et al. 2004). However, few studies paid attention to the importance and effects of protein on biohydrogen production. Bai et al. (2004) used different ratios of glucose and peptone as multiple substrates to investigate the roles played by carbohydrate and protein in hydrogen fermentation from artificial wastewater and demonstrated that suitable ratios of glucose and peptone improved the growth of hydrogen producing bacteria. Many agricultural wastes, industrial and domestic food wastes contain starch, which is the most appropriate organic sources for biohydrogen production. Starch containing solid waste is easier to process for carbohydrate and hydrogen gas formation. Complex nature of such solid wastes may affect the attempted biohydrogen production. For example, food wastes contain carbohydrates and certain amount of proteins. Lay et al. (2003) investigated the influence of the chemical nature of high-solid organic wastes (HSOW) on biohydrogen generation. In their study, rice and potato used as carbohydrate-rich HSOW had 20 times larger the hydrogen-producing potential than those of egg and lean meat used as protein-rich HSOW. In real practices of solid waste fermentation, hydrogen fermentation reactors may receive different types of organic wastes from time to time. The fluctuation of protein content in the feed may impose different effect on the biohydrogen production from the carbohydrate content in the same feed. The understanding on the extent of protein effect can provide vital information on reactor design and process operation of biohydrogen production. This paper discusses the influence of protein content on the biohydrogen production from carbohydrates, especially from starch in the same organic source. For simplicity, rice as starch source and soybean residue as major protein source were mixed to achieve various combinations of starch and protein contents.
The biohydrogen production from different mixtures was evaluated to understand the effect of protein on biohydrogen production.

**MATERIALS AND METHODS**

**Experimental design**

To harvest anaerobic spore-forming hydrogen-producing clostridia, anaerobic sludge taken from a continuously operated 100 L methanogenic bioreactor was used (Wang et al. 2005). It was heated for 2 h at 103°C to inactivate hydrogen consumers (Lay et al. 1999). The experiments were performed at 37°C using the 250-mL glass bottles as lab bioreactors. A water bath with orbital shaker was used to maintain temperature and provide shaking at 50 rpm. Constant pressure manometer (BS ISO 14853:2005) was adopted to measure the volume of biogas. Characteristics of rice and soybean residues used as starch-rich and protein-rich substrates, respectively, are shown in Table 1. Rice and soybean residues were oven dried at 103°C for 24 h, and then were ground to particles with size less than 0.2 mm. The mixtures of rice and soybean residue were prepared to obtain different ratios of starch and protein (w/w): 11.7 (M01), 11.4 (M02), 10.0 (M03), 8.8 (M04), 4.5 (M05), 2.8 (M06), 1.7 (M07), 0.6 (M08), 0.4 (M09), 0.3 (M10), and 0.2 (M11). 200 ml of 5% slurries were prepared from every substrate mixture and placed in lab bioreactors. No rice and soy bean were added into M12 used as biotic control.

Mineral medium with the composition, g/L: 200 NH4HCO3, 100 KH2PO4, 10 MgSO4·7H2O, 1.0 NaCl, 1.0 Na2MoO4·2H2O, 1.0 CaCl2·2H2O, 1.5 MnSO4·7H2O, and 0.278 of FeCl2, was prepared (Bahl et al. 1986). 1 ml of mineral medium, 50 ml heat-shocked anaerobic and 0.07M phosphate buffer (Oh et al. 2003) were added to each lab bioreactor. pH was initially adjusted to 6.5 with 1.0 N NaOH or 1.0 N HCl and allowed to drop during batch fermentation.

**Analytical methods and data analysis**

Gas composition was analyzed by a HP5890A Gas Chromatograph (GC) (Hewlett Packard) equipped with a thermal conductivity detector and a stainless-steel column packed with Hayeseq Q (80/100 mesh). Argon was used as a carrier gas. Ammonia was determined by Flow Injection Analysis using QuickChem Automated Ion Analyzer (Lachat, USA). Dissolved carbohydrate was determined by Phenol–Sulfuric Acid Assay (Dubois et al. 1951). Dissolved protein and amino acid were determined by Bradford Assay (Bradford 1976). Protein contents in solid samples were estimated by multiplying the solid nitrogen content with a factor of 5.7 (Owusu-Apenten 2002). Starch contents in the solid were determined by Perchloric Acid Immersion Method plus Anthrone–Sulfuric Acid Assay (Viles & Silverman 1949).

A modified Gompertz Equation (Zwietering et al. 1990) was used to fit the cumulative hydrogen production curves for each bioreactor to obtain the biohydrogen production potential $P$, the hydrogen production rate $R$ and lag phase $\lambda$ (Lay et al. 1998)

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

where $H$ is the cumulative hydrogen production (ml) at time $t$ (h), $\lambda$ is the lag-phase time (h), $P$ is the hydrogen production potential (ml), $R_m$ is the maximum hydrogen production rate (ml/h), $t$ is the incubation time (h), $e$ is exp(1) = 2.718. Parameters ($P$, $R_m$ and $\lambda$) were estimated according to Lay et al. (1998). The specific hydrogen production potential, $P_s$ (ml/g starch as glucose), was obtained by dividing $P$ by starch content (g as glucose) of the food waste in each bioreactor. The specific hydrogen

### Table 1 | Characteristics of rice and soybean residue

<table>
<thead>
<tr>
<th></th>
<th>VS</th>
<th>C</th>
<th>N (% of total solid)</th>
<th>Protein</th>
<th>Starch</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rice</td>
<td>89.4 ± 0.5</td>
<td>48.2 ± 0.7</td>
<td>1.3 ± 0.2</td>
<td>7.3</td>
<td>86.0 ± 0.3</td>
</tr>
<tr>
<td>Soybean residue</td>
<td>94.6 ± 0.8</td>
<td>54.8 ± 0.2</td>
<td>4.5 ± 0.5</td>
<td>25.5</td>
<td>6.3 ± 0.2</td>
</tr>
</tbody>
</table>
production rate, $R_s$ (ml/h g-VSS), was obtained by dividing $R_m$ by VSS added. Hydrogen conversion efficiencies for different substrates were compared based on $P_s$ and $R_s$.

RESULTS AND DISCUSSION

Biohydrogen production

The specific hydrogen production potential was plotted against starch/protein ratio in Figure 1. The amount of hydrogen produced, which was plotted as the reference, seemed to be constant around 450 ml within the starch/protein ratio range of 1.7 to 11.7. However, if expressed as specific hydrogen production potential, the maximum biohydrogen production efficiency of 0.99 mol H$_2$/mol initial starch as glucose was achieved at the starch/protein ratio of 1.7. In the same figure, the maximum specific hydrogen production rate of 530 ml H$_2$/h g-VSS was found at the starch/protein ratio of 1.7.

Soluble protein and ammonia

Readily available organic nitrogen, such as dissolved proteins and amino acids resulted from anaerobic degradation, directly meets the nitrogen requirement for the microorganism growth. Brosseau et al. (1986) observed that hydrogen production and cell growth occurred simultaneously. Bai et al. (2004) reported that optimal hydrogen production and cell growth were both observed in the fermentation of one of the multiple liquid substrate combinations, 3 g-COD/L glucose plus 2 g-COD/L peptone. Relative high protein content could enhance the biohydrogen production through the direct organic nitrogen supply to microorganisms. In Figure 2a, the soluble protein concentration in M07 at starch/protein of 1.7 was maintained at 200–250 mg/L for 20 h, while the concentration in M01 at highest starch/protein ratio of 11.7 was much lower and nearly the same as the control in M12. The microorganisms in M01 and M12 might need to synthesize necessary organic nitrogen matter first from inorganic nitrogen ammonia. Although the medium containing ammonia as inorganic nitrogen at around 400 mg NH$_4^+$–N/L has been provided equally to each bioreactor, in Figure 2b ammonia concentration in M01 decreased from 400 to 200 mg NH$_4^+$–N/L in 45 h. In contrast, M07, with starch/protein ratio of 1.7 had a stable ammonia concentration above 600 mg NH$_4^+$–N/L in the whole process. Therefore the advantage of readily available organic nitrogen was obvious. The same conclusion was drawn in Bai et al. (2004).

pH development

The protein content also affected the biohydrogen production through the influence on pH change. All the experiments were stopped at the 45th h when there was no more biohydrogen production. The final pH values and
calculated pH changes (negative sign) are plotted in Figure 3a. M01 through M06 experienced a significant pH decrease of roughly 2 units. Meanwhile, M08 through M11 experienced less pH decrease, and the final pHs in these bioreactors were higher than 6. The final pH in M07 was around 5 and experienced moderate pH decrease of 1.5 pH units.

Organic nitrogen in proteins is transformed into inorganic ammonia nitrogen in anaerobic degradation. Ammonia and amino groups released from proteins could maintain suitable pH by alkalinity produced:

\[
R \quad \text{NH}_2 \rightarrow \text{NH}_3^+ + \text{HCO}_3^- + x\text{CO}_2 + y\text{H}_2\text{O}
\]  

(2)

The potential pH decrease imposed by volatile fatty acids was buffered by the bicarbonate produced. As long as the biodegradation of protein accompanies the overall organic biodegradation, the pH could be stabilized at a certain level. Such equilibrium can be prospected by evaluating the protein/starch ratio. In Figure 3b, it is found that to maintain pH decrease within 0.5 pH unit, the protein/starch has to be greater than 2. Lay (2000) demonstrated that pH-window for optimal hydrogen production from carbohydrates may be so narrow that half unit decrease in pH may cause a 50% decrease in hydrogen production from the optimum.

**Soluble carbohydrate**

The pH profiles of M01, M7, and M11 are plotted in Figure 4a. The steepness of pH profiles during the decrease was correlated with starch/protein ratio. pH in
M01 decreased dramatically to 5.0 in 15 h while pH in M07 decreased gently to below 5.0 in 40 h. The approximate rates of pH decrease in M01 and M07 were 0.10 unit/h and 0.04 unit/h, respectively. High starch/protein ratio resulted in steep pH decrease due to accumulation of acids produced which were not buffered by abovementioned acid–base equilibrium. The biohydrogen production from soluble carbohydrate was thus inhibited. In Figure 4b, the soluble carbohydrate in M01 is accumulated significantly from 3,004 mg/L at the 11th h to 17,140 mg/L at the 16th h in 5 h. This accumulation responded to a severe pH decrease in M01 from pH of 6.1 to 5.0 concomitantly (Figure 4a). In distinct contrast, the soluble carbohydrate in M07 was maintained at 1,400 mg/L due to a steady pH decrease throughout the whole process and its accumulation did not occur. Apparently the equilibrium between soluble carbohydrate production (hydrolyzed from solid) and consumption (converted to hydrogen and acids) was achieved at the starch/protein ratio of 1.7.

CONCLUSIONS

This study demonstrated some important effect of protein on biohydrogen production from starch. It was found the maximum specific hydrogen production potential, 0.99 mol H₂/mol initial starch as glucose, and maximum specific hydrogen production rate, 530 ml H₂/h g-VSS, occurred at a starch/protein ratio of 1.7. The presence of protein in the food waste not only provided buffering capacity to neutralize the volatile fatty acids as concurrent products, but also enhanced the production by providing readily available organic nitrogen in the form of soluble protein and amino acids to microorganisms. The existence of protein in the substrate of biohydrogen production is important. In the real situation of biohydrogen production from solid food waste, the organic protein content in feedstock should be optimized to achieve the maximum possible amount of hydrogen from carbohydrates.

REFERENCES


