Biohydrogen Enhancement Using Highly Porous Activated Carbon
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ABSTRACT: Treated activated carbon (T-AC) was found to be highly effective for fermentative hydrogen production due to its highly porous structure. The addition of T-AC at 8.3−33.3 g/L in batch experiments increased the hydrogen production rate and yield from 0.8 to 1.8 mL of H2/l and from 1.24 ± 0.13 to 2.60 ± 0.21 mol of H2/mol of sucrose, respectively. The high activity of T-AC was attributed to a greater porosity and active sites as compared to commercial activated carbon. The use of T-AC reduced the inhibitory effect of butyric acid with selective adsorption of butyric acid over metabolite products. Moreover, T-AC showed a highly durable performance for three repeated cycles for hydrogen production. The hydrogen production increased by 73% as compared to the control for the first cycle and dropped by 32% after three consecutive cultivation cycles. It is postulated that a highly porous surface and affinity of T-AC may provide sites for volatile fatty acids adsorption, thereby reducing the inhibitory effect on hydrogen-producing bacteria.

1. INTRODUCTION

Hydrogen is one of the most promising energy sources for the future. Burning of hydrogen for energy yields only water and, hence, does not contribute to environmental pollution. There are many techniques for producing hydrogen, including steam reformation of natural gas, coal gasification, and splitting water with electricity, typically generated from fossil fuels.1 Hydrogen can also be produced biologically. It is the most environmentally friendly method for hydrogen production because hydrogen can be produced from raw materials such as organic wastes at ambient temperatures and pressure.2−5 Dark fermentation is one of the attractive options to produce hydrogen through microbial fermentation. This process does not require external energy to drive the process or a large surface area to capture the necessary light for photosynthesis. Moreover, it can utilize cheap organic substances and it is relatively simple and inexpensive to operate. However, dark fermentation still requires further development to achieve both high yields and low costs for it to be of practical use.

During a fermentation process, hydrogen is associated with acetic, butyric, propionic, and lactic acids, and ethanol as the fermentation end products. The production and accumulation of these soluble metabolites has significantly affected hydrogen production in mixed culture systems. Many studies found that, at a high concentration, these soluble metabolites can inhibit growth of hydrogen-producing bacteria and then inhibit the fermentative hydrogen production accordingly. The inhibitory effect of butyric acid concentrations on hydrogen production reported in the literature ranges from 25 to 300 mmol/L. Van Ginkel and Logan6 found that addition of acids (25 mmol/L) decreased hydrogen yields by 13% for acetic acid and 22% for butyric acid. A concentration of 60 mmol/L of either of these acids decreased hydrogen production greater than 93%. Wang et al.7 found that hydrogen yield and hydrogen production rate decreased with an increase in ethanol, acetic acid, propionic acid, and butyric acid concentrations from 0 to 300 mmol/L. Zhang et al.8 also reported a decrease in hydrogen production with an increased dosage of acetate and butyrate that ranged from 0 to 500 mmol/L and from 0 to 250 mmol/L, respectively. The inhibitory effect of acetate on fermentative hydrogen production was less significant as compared to that of butyrate.9 In a mixed culture system, Zheng and Yu,9 found that butyrate affected acidogenesis of glucose, thereby lowering hydrogen production. The hydrogen yield decreased from 1.85 to 0.32 mol of H2/mol of glucose with a change of butyrate concentration from 4.18 to 284.66 mmol/L. The potential inhibition of cells caused by these soluble metabolites is a major concern in the hydrogen fermentation process. Thus, improvements of the bioactivity of hydrogen-producing bacteria as well as the avoidance of end product inhibition are needed for an increase in hydrogen productivity. However, there is little information concerning the avoidance of end product inhibition for biological hydrogen production. There are a few studies on adsorbents and their adsorption behavior of fatty acids, including acetic, propionic, and butyric acids.10 Activated carbon presented a higher adsorption capacity as compared to other adsorbents reported. The polar characteristic of fatty acids decreases with an increase in the length of the nonpolar hydrocarbon chain, thereby improving the affinity between the activated carbon (nonpolar adsorbent) and the acids. Aljundi et al.11 reported the adsorption of lactic acid from fermentation broth onto silicalite molecular sieves. Silicalite showed a high adsorptive capacity, up to 37 g/kg in broth. This study researched treated activated carbon (T-AC) due to its relatively large surface area and low cost. T-AC may provide an alternative solution to overcome undesirable volatile fatty acids (VFAs) accumulation during the fermentation process.

2. MATERIALS AND METHODS

2.1. Materials. T-AC was used as an adsorbent in batch fermentation to promote biohydrogen production. It was obtained from the National Nanotechnology Center, Thailand. It was prepared from coconut shells, having a granular shape with an average pore diameter of approximately...
0.7 nm. Its characterizations were investigated and reported by Wongsarivej et al.12 Prior to use, the T-AC was sieved to a particle size of 1180 μm. Relatively the same size of commercial activated carbon (C-AC) was used for comparative study. The morphology of activated carbon was examined before and after fermentation using scanning electron microscopy (SEM).

2.2. Inoculum and Medium. The anaerobic sludge was obtained from the bottom portion of an upflow anaerobic sludge blanket reactor (UASB) treating brewery wastewater in Thailand. The characteristics of anaerobic sludge are given in Table 1. The sludge was heat-treated at 105 °C for 30 min and used as inoculum in the hydrogen fermentation tests to remove methanogenic activity. Sucrose solution was prepared freshly before use by dissolving 20 g of sucrose in 1 L of distilled water and used as a substrate with a concentration of 24 g of COD/L. The substrate was supplemented with a sufficient amount of inorganic constituents for bacterial growth. The nutrient solution was composed of (mg/L): NH₄HCO₃, 160; KH₂PO₄, 80; FeCl₃·4H₂O, 70.5; NaCl 0.4; MgSO₄·7H₂O 4; CaCl₂·2H₂O 0.4; MnSO₄·7H₂O 0.6; and Na₂MoO₄·2H₂O 0.4.13

2.3. Experimental Procedures. Batch tests were carried out to investigate the effect of different types of activated carbon consisting of C-AC and T-AC for enhancement of hydrogen production. Different doses of activated carbon in the range of 8.3—33.3 g/L were added into 10 mL of heat-treated sludge and enriched with 45 mL of synthetic medium, together with 5 mL of essential growth nutrients in 120 mL serum bottles with a working volume of 60 mL. The initial pH of 5.5 was adjusted using 1 N NaOH or 1 N HCl. After cultivation, the suspensions were sparged with nitrogen gas for 3 min to create anaerobic conditions. The bottles were placed in an incubator shaker under mesopholic conditions at 37 °C and shaken at 90 rpm. Seed sludge without activated carbon addition was used as a control. The experiments were performed in triplicate. For the T-AC recyclability study, after 8 days of cultivation, 50 mL of supernatant was withdrawn, resulting in a remaining volume in the bottle of 10 mL. Fresh medium containing 45 mL of synthetic medium and 5 mL of essential growth nutrient were added, and the fermentative investigation was repeated. The T-AC was used for three consecutive cycles. The amount of evolved gas was measured at room temperature by syringes. The gas composition was determined by a gas chromatograph (GC). At the end of cultivation, mixed liquor samples from each serum bottle were drawn and analyzed for COD, volatile fatty acids, and pH.

2.4. Chemical Analysis. The characteristics of activated carbons were examined by SEM and Brunauer–Emmet–Teller (BET) analysis at the National Nanotechnology Center, Thailand. The activated carbons were dried in an oven at 60 °C for 2 h before their SEM analysis. The functional groups of samples were analyzed by the Fourier transform infrared spectroscopy (FTIR) spectra. FT-IR spectra were recorded on a Nicolet 6700 spectrophotometer at ambient temperature using a potassium bromide (KBr) disk method. The samples were prepared from disks containing 0.001 g of sample and 0.25 g of KBr. The spectra were recorded at 2 cm⁻¹ resolution with a total of 32 scans with wavenumbers ranging from 400 to 4000 cm⁻¹. The compositions of biogas were analyzed by a gas chromatograph (PerkinElmer, USA) fitted with a Porapak Q column. Helium gas was used as the carrier gas at a flow rate of 25 mL/min. The operating temperatures of column, detector, and injector were 45, 100, and 100 °C, respectively. VFAs, including acetic, butyric, and lactic acids, were analyzed by a high-performance liquid chromatography (HPLC) (Agilent 1200 infinity Series) equipped with an OA column at 35 °C. Sulfuric acid (0.01 N) was used as the mobile phase at a flow rate of 0.8 mL/min. The aqueous samples were filtered through a 0.2 μm Millipore membrane before analysis with an injection volume of 20 μL. The pH value was measured by a pH meter (METTLER TOLEDO, SevenGo SG2). Sucrose concentration and the properties of the anaerobic sludge, including COD, TSS, and VSS, were determined in accordance with the procedures described in Standard Methods for the Examination of Water and Wastewater.14

2.5. Kinetic Modeling. In order to describe the progress of the fermentative hydrogen production process, the modified Gompertz model was used to fit data of the cumulative hydrogen production as shown in eq 1

\[ H = H_{\text{max}} \exp\left(-\exp\left(\frac{R_n \cdot e^{(\lambda - t)} + 1}{H_{\text{max}}}\right)\right) \]  

where \( H \) and \( H_{\text{max}} \) denote the cumulative hydrogen production (mL) and the maximum cumulative hydrogen production (mL), respectively. \( R_n \) represents the hydrogen production rate (mL/h), and \( \lambda \), \( t \), and \( e \) are the lag-phase time (h), the cultivation time (h), and the Euler’s constant (2.718), respectively.

3. RESULTS AND DISCUSSION

3.1. Activated Carbons for Enhancement of Biohydrogen Production. Different types of activated carbon, namely, C-AC and T-AC, were examined to identify the roles of activated carbon for enhancement of hydrogen production. Hydrogen production tests were carried out at different activated carbon doses of 8.3—33.3 g/L and analyzed for their hydrogen production kinetics. The cumulative hydrogen productions obtained for each dose were fitted with the modified Gompertz equation as described in eq 1. The result showed that the addition of activated carbon in the fermentation broth can markedly enhance hydrogen production during a fermentation period of 300 h (Figure 1). The maximum cumulative hydrogen production increased linearly with an increase in activated carbon dose, meaning that there was no substrate limitation in the system within the experimental period (Figure 1a). With the addition of activated carbon at 33.3 g/L, the maximum cumulative hydrogen production was 2.3 and 1.7 times the control for T-AC and C-AC, respectively. However, an increase in activated carbon dosage is not in proportion to the rate of hydrogen production (Figure 1b). There was probably an increase in partial pressure due to a greater amount of hydrogen gas produced, relative to the amount of activated carbon added, thereby causing a lowering of hydrogen production rate.15 In actual operation, the limitation of hydrogen production rate can be easily alleviated by frequent and regular withdrawal of hydrogen gas from the system. As shown in Figure 1c, the hydrogen yield increased sharply from 1.24 ± 0.13 to 2.60 ± 0.21 mol of H₂/mol of sucrose with an increase in T-AC dose, while a lower increment of hydrogen yield was observed from C-AC. In comparison to C-AC, T-AC exhibited a higher activity for fermentative hydrogen production. The possible explanation for an increase in activity of T-AC due to VFAs adsorption is discussed in section 3.3.

3.2. Characterization of Activated Carbon. A highly porous surface area of T-AC is evident through the SEM images (Figure 2) and BET analysis (Table 2). C-AC showed less surface roughness with larger pore size (Figure 2 (a-1) and (a-2)) as compared to T-AC (Figure 2 (b-1) and (b-2)). The BET surface area and total pore volume of T-AC were reported12 at 1154 m²/g and 0.49 cm³/g, which is much higher, as compared to commercial activated carbons.16 This leads to greater active sites.
and areas, which is beneficial to fermentative hydrogen production.

To further understand the difference in properties of C-AC and T-AC, FTIR was used. The fundamental frequencies of C-AC and T-AC and their respective possible band frequencies in the FTIR spectrum are presented in Table 3. The functional groups O=H, C−H, C=O, and C−O were observed on the surface for both activated carbons. However, no peaks due to adsorbed CO₂ were detected in spectra of T-AC. The relatively weak absorption band in FT-IR spectra at 2359.6 cm⁻¹ might be due to the adsorbed atmospheric carbon dioxide at the surface of C-AC. The absorption peaks of CO₂ were possibly diminished during the carbonization and activation processes of T-AC.

3.3. Soluble Metabolite Distribution. To further clarify the beneficial use of T-AC on biohydrogen production, the time course of hydrogen production, pH, and soluble metabolite distribution are plotted in Figure 3. VFAs detected in the culture system for the T-AC were acetic acid, butyric acid, and lactic acid. The sucrose was consumed gradually while acetic and butyric acids were produced as the main metabolic products, relatively in low concentration, during the initial period of fermentation. Hydrogen and acetic acid continued to be produced until approaching the critical pH of 3 (approximately 96 h). After that, a metabolic shift to lactic acid occurred. The hydrogen production declined after 160 h, caused by sucrose limitation, pH reduction, and lactic and butyric acids accumulation. As compared to the control, a higher rate and amount of acetic acid were found and then utilized, resulting in a higher hydrogen production. The result indicated that production of acetic acid favors the production of hydrogen. The concentration of acetic acid varied in a narrow range of 18 mmol/L after 160 h of fermentation (Figure 3 (a2)). After that, lactic acid was observed at 45 and 42 mmol/L for the control and T-AC tests, respectively. It is noted that the concentration of butyric and lactic acids observed in the control significantly reduced the efficiency of hydrogen fermentation. It is noteworthy to mention that the concentration of acetic acid, as observed in T-AC tests at 18 mmol/L was lower than inhibition levels found by many researchers.⁶⁻⁸ The concentration of butyric acid at 22 mmol/L observed in the control was within the inhibition levels.⁹ In this study, hydrogen yield was reduced by 52%, at 22 mmol/L of butyric acid. A change in the main fermentation pathway to lactic acid was found when the pH value was below 3 (Figure 3 (a1) and (b1)). At this pH range, hydrogen production significantly decreased. Therefore, the accumulation of metabolite products should be avoided.

In order to understand the mechanism of T-AC for enhancement of hydrogen production, the adsorption kinetics of VFAs on T-AC was investigated (Figure 4). The adsorption of butyric acid onto T-AC was quite rapid during the first 5 h and then became slower after 9 h. A similar result was observed for lactic and acetic acids, but lower concentrations of lactic and acetic acids were adsorbed onto T-AC. The maximum adsorption capacity of 8 mM/g of T-AC was found for butyric acid after 48 h of adsorption test. In actual fermentation, a slightly lower amount of butyric acid, at 7 mM/g of T-AC, was adsorbed over 300 h of fermentation. This was based on the calculation on the different amount of butyric acid found in T-AC as related to the control. The initial faster rate was attributed to the availability of fresh active sites on the surface of the T-AC. This result indicates that the adsorption behavior of T-AC was favored over the butyric acid, followed by acetic and lactic acids. This behavior is due to the fact that activated carbon is a nonpolar adsorbent.
An increase in the length of the nonpolar hydrocarbon chain decreases the polar characteristic of fatty acids, thereby improving the affinity between the activated carbon and the acids.\(^{10}\)

Because of a special treatment given to the material by the manufacturer, the surface areas and active sites were increased significantly, as evidenced by the SEM analysis (Figure 2). The treatment of T-AC has no significant effect on the functional surface groups of the carbon, as reported in Table 3. Thus, metabolic adsorption of T-AC depends mainly on the porosity and electrostatic interaction between charged solutes and the surface of the carbon. The additional accessible surface area and active sites increased the potential of metabolites adsorption to reduce acids within the aqueous phase, particularly for butyric acid. This, thereby, reduced the accumulation of butyric acid in the aqueous phase and slowed down a decrease of pH, which is in favor of acetic acid but not the lactic acid pathway. As a result, more hydrogen production was gained. T-AC can be further developed as an alternative support material for nanoactive catalysts, especially hydrotalcite catalysts, as reported in our previous study.\(^{18,19}\)

### Table 2: Physical Properties of Different Materials

<table>
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<tr>
<th>adsorbents</th>
<th>surface area (BET) (m(^2) · g(^{-1}))</th>
<th>mesopore volume (cm(^3) · g(^{-1}))</th>
<th>pore diameter (Å)</th>
<th>function</th>
<th>ref</th>
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<td>VFAs adsorption</td>
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<td>regenerated clay</td>
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<td>36.43</td>
<td>VFAs adsorption</td>
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<td></td>
<td>bacterial immobilization</td>
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<tr>
<td>granular AC</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>pure silica SBA-1S</td>
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<td>1.61</td>
<td>96.3</td>
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<td>granular T-AC</td>
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<td>0.49</td>
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<td>VFAs adsorption</td>
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<tr>
<td>granular C-AC</td>
<td>761</td>
<td>0.37</td>
<td>20</td>
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### Table 3: Functional Surface Groups Presented on C-AC and T-AC

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<th>band position (cm(^{-1}))</th>
<th>possible assignments</th>
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<tr>
<td>3431.6</td>
<td>hydroxyl group (O−H stretching)</td>
</tr>
<tr>
<td>2920.0</td>
<td>alkane (C−H sp(^3) stretching)</td>
</tr>
<tr>
<td>2359.6</td>
<td>CO(_2) (CO(_2) stretching)</td>
</tr>
<tr>
<td>2078.0</td>
<td>CO (CO stretching)</td>
</tr>
<tr>
<td>1734.7</td>
<td>carboxylic acids (C=O stretching)</td>
</tr>
<tr>
<td>668.0</td>
<td>CO(_2) (CO(_2) bending)</td>
</tr>
</tbody>
</table>

Figure 3. Time course of hydrogen production and pH (1) as well as substrate and metabolite distribution (2) for T-AC (a) and control (b).

Figure 4. Adsorption kinetics of VFAs on T-AC.
material and nаноactive catalyst were found to be beneficial to biohydrogen production and also for the reuse of catalyst.

The adsorption mechanism of AC can be understood on the basis of pore diameter, the functional groups on the surface of carbon, and electrostatic interaction between charged solutes and the surface. A large amount of butyric acid adsorbed on the surface area was attributed to electrostatic interaction between metabolite and the surface of the carbon. In this study, the pH of the fermentation broth was at 5.5, which is below the pH of zero charge of the activated carbon, as reported by Montane et al. at 8–8.7. This leads to a positive charge distributed on the surface of the carbon among the basic functional groups, thereby decreasing in the strength of the electrostatic interactions between the positive surface and the negatively charged acids containing dissociated acetyl groups.

### 3.4. Recyclability of T-AC for Biohydrogen Production.

The stability of the T-AC for hydrogen production was investigated in three repeated fermentative cycles based on fresh substrate for each cycle and the same T-AC, at 33.3 g/L. The hydrogen production for three consecutive cycles is described in Figure 5. After three cycles of use, the ability of T-AC to enhance biohydrogen production was reduced by 32%, as compared to the control. Nevertheless, the amount obtained was still higher than that of the control without T-AC. As can be seen in Table 4, the Gompertz model was applied to the cumulative hydrogen production. The model fits the data well with an R² value of 0.999. The hydrogen production kinetics were affected by repeated fermentative cycles. A significant difference in hydrogen yield for the first cycle was observed as compared to the second and third cycles (p < 0.05). However, there is no significant difference observed for the hydrogen yield between the second and third cycles (p > 0.05). In addition, the maximum cumulative hydrogen production and lag phase did not show a significant effect between the second and third cycles. Nevertheless, the rate of production for the third cycle was significantly reduced. This result indicated that the repeated use of T-AC is useful with prolonged fermentation. In the repeated fermentative batch, the cells can remain onto the T-AC surface and establish hydrogen-producing capability. When the fermentation was coupled to a simultaneous adsorption capacity of VFAs, butyric acid was adsorbed, and the levels of butyric acid were maintained below the inhibitory level.

The decrease in the T-AC activity can be explained by the saturation of T-AC. It is observed that a higher concentration of VFAs was obtained during the second and third cycles. Although saturation capacity is reached, T-AC still acts as a support for immobilized bacteria. It is clearly observed that the combination effect of bacteria immobilization and VFAs adsorption on the porous support of T-AC has improved hydrogen production. A lower hydrogen yield was consistent with the removal of COD, which was highest for the first cycle at 84%, and reduced to 62% in the third cycle (data not shown).

### 4. CONCLUSION

A highly porous surface area was found to support the activity of T-AC for hydrogen production, resulting in an increase in hydrogen yield as compared to C-AC. The maximum hydrogen yield was 2.60 ± 0.21 mol of H₂/mol of sucrose. T-AC can be reused repeatedly at least for three cultivation cycles with a slight decrease in performance. The adsorption capacity for butyric acid is 8 mM/g of T-AC. The favorable adsorption of butyric acid has a beneficial effect on biohydrogen production, which is due to the prevention of accumulation of butyric acid in the aqueous phase.

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**Notes**

The authors declare no competing financial interest.

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#### REFERENCES


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**Table 4. Estimated Value of Parameters by the Modified Gompertz Equation and Hydrogen Yield**

<table>
<thead>
<tr>
<th>run no.</th>
<th>H₂ yield (mol of H₂/mol of sucrose)</th>
<th>Gompertz model</th>
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<tr>
<td>1</td>
<td>2.60 ± 0.21</td>
<td>147.1</td>
<td>2.00</td>
<td>18.53</td>
<td>0.9995</td>
</tr>
<tr>
<td>2</td>
<td>1.58 ± 0.57</td>
<td>95.9</td>
<td>2.15</td>
<td>21.66</td>
<td>0.9994</td>
</tr>
<tr>
<td>3</td>
<td>1.64 ± 0.39</td>
<td>102.6</td>
<td>1.24</td>
<td>20.83</td>
<td>0.9995</td>
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