Producing hydrogen from wastewater sludge by Clostridium bifermentans

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Received 16 August 2002; received in revised form 9 December 2002; accepted 11 December 2002

Abstract

Excess wastewater sludge collected from the recycling stream of an activated sludge process is biomass that contains large quantities of polysaccharides and proteins. However, relevant literature indicates that the bio-conversion of wastewater sludge to hydrogen is limited and therefore not economically feasible. This work examined the anaerobic digestion of wastewater sludge using a clostridium strain isolated from the sludge as inoculum. A much higher hydrogen yield than presented in the literature was obtained. Also, the effects of five pre-treatments—ultrasonication, acidification, sterilization, freezing/thawing and adding methanogenic inhibitor—on the production of hydrogen were examined. Freezing and thawing and sterilization increased the specific hydrogen yield by 1.5–2.5 times to that of untreated sludge, while adding an inhibitor and ultrasonication reduced the hydrogen yield.

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Keywords: Hydrogen; Sludge; Fermentation; Pre-treatment

1. Introduction

Hydrogen is a clean source of energy. The anaerobic conversion of biomass has been demonstrated as a technically feasible way of generating hydrogen. The substrates used include high-strength wastewater (Bolliger et al., 1985; Liu et al., 1995; Ueno et al., 1996; Zhu et al., 1999), solid waste (Lay et al., 1999; Mizuno et al., 2000b) or certain aqueous solutions, such as those of molasses (Tanisho and Ishiwata, 1994), glucose (Kataoka et al., 1997; Lin and Chang, 1999), crystalline cellulose (Lay, 2001), peptone (Bai et al., 2001) and starch (Lay, 2000). Various ways of enhancing hydrogen production have been reported (Tanisho and Ishiwata, 1995; Sparling et al., 1997; Tanisho et al., 1998; Liang et al., 2001; Mizuno et al., 2000a). During anaerobic digestion, methanogenic or sulfate-reducing bacteria consume hydrogen produced by acidogenic bacteria, contributing negatively to bio-hydrogen production (Mizuno et al., 2000a). Effectively extracting hydrogen from an anaerobic reactor depends on special procedures to block out the co-metabolic chains (Adams and Stiefel, 1998). A low partial pressure of hydrogen in the vapor phase promotes

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acidogenic dehydrogenation and methanogenesis (Guwy et al., 1997; Gurijala et al., 2000; Mizuno et al., 2000a).

Table 1 lists some results from the literature. Various means of producing hydrogen using numerous substrates and seed bacteria have been reported, such as g-H₂/g-VSS (volatile suspended solids), ml-H₂/g-COD (chemical oxygen demand) and others. Experimental results can almost never be compared because details for unit conversion are unavailable. Certain assumptions were made to convert the data presented in Table 1 for comparison. The production of bio-hydrogen mostly ranges from 5 to 10 mmol-H₂/g-dried solids (DS) for glucose and 1–1.5 mmol-H₂/g-DS for protein-containing water.

Waste-activated sludge from a wastewater treatment plant contains high levels of organic matter and thus are a potential substrate for producing hydrogen. Very few studies have addressed this topic. Limited data show that the bio-hydrogen yield using waste activated sludge and anaerobic fermentation is rather low at ≈ 0.08 mmol-H₂/g-DS (Huang et al., 2000). Pre-treatment can increase the efficiency of anaerobic stabilization of sludge by hydrolyzing the insoluble organic matter to water (e.g. see Lee and Mueller (2001) and the references cited therein). The use of thermal boiling to hydrolyze sludge has been demonstrated to promote the anaerobic production of methane (Chu et al., 2002a). Cheng et al. (2000) reported a yield of 0.7 mmol-H₂/g-COD by anaerobically fermenting the thermally boiled waste-activated sludge. This value is close to that obtained for fermenting protein-containing wastewater, but is still much lower than that for polysaccharides.

Cheng et al. (2000) also noted a time lag of 66 h for the microbes to adapt themselves to produce hydrogen from the boiled sludge. The production of hydrogen from waste sludge does not seem very promising, given the low yields reported in these preliminary studies.

Systematic studies of the anaerobic fermentation of wastewater to produce hydrogen are still lacking. This work used clostridium strains isolated from the collected wastewater sludge as an inoculum to produce hydrogen, using the sludge itself as the substrate. Apart from the thermal boiling employed by Cheng et al. (2000) to pre-treat the sludge, the effects of common pretreatment processes on hydrogen conversion are yet to be examined, including treatment with acid (Jean et al., 2000), ultrasound (Chu et al., 2002b) and freezing and thawing (Chu et al., 1999). The impact of five different sludge pre-treatment processes on hydrogen yields is examined here.

2. Experimental

2.1. Substrate

Wastewater sludge was taken from the recycled stream of the secondary treatment stage of Min-Sheng Municipal Wastewater Treatment Plant in Taipei, which handles 15 000 t wastewater daily. The collected sample settled under the influence of gravity to yield a solids content of 16 500 mg l⁻¹ and was the substrate in testing. The pH value of the sludge was ≈ 6.4. The chemical oxygen demand (COD) of the sludge was 24 800 mg l⁻¹, measured directly from a spectrometer (DR/2000, Hach, USA). The COD of the filtrate of the sludge sample obtained after filtering through a 0.45-mm membrane, called soluble COD (SCOD), was 339 mg l⁻¹ for the original sludge. The elemental compositions of the dried sample were C: 34.2%, H: 5.3% and N: 5.4%, obtained using an elemental analyzer (Perkin-Elmer 2400 CHN). The alkalinity of the suspension was measured at 325 mg l⁻¹ of CaCO₃, using standard method APHA SM 2320 (APHA, 1992). A strong pH-buffering effect was expected to be exhibited by the collected sludge.

2.2. Pre-treatment

Five pre-treatments were applied. These pre-treatments not only released insoluble organic matter into water to increase the efficiency of fermentation, but also inactivated methanogenic bacteria in the substrate to reduce their consumption of hydrogen.

1) Ultrasonication—sonication tests were performed with the help of a cell-breaker (SONICATOR XL-2020, Heat System-Ultrasonics,
Inc.) at a frequency of 20 kHz. Original sludge (300 ml) was placed in a 500-ml beaker with the ultrasonic probe positioned 2 cm above the bottom of the beaker. The sonication time was 20 min to release the insoluble organic matter from the solids (Chu et al., 2001).

2) Acidification—perchloric acid (HClO₄) was mixed with the sludge sample for 10 min to alter the pH of the suspension to 3. Then, the sample was stored at 4°C for 6 h (Jean et al., 2000).

3) Sterilization—samples of sludge were sterilized at 121°C and 1.2 kgf cm⁻² (Huxley Autoclave, HL-360) for 30 min.

4) Methanogenic inhibitor—BESA (C₂H₄BrO₃SNa, sodium 2-bromoethanesulfonate, Sigma, USA) has a chemical structure (BrCH₂CH₂SO₃⁻) that resembles that of the co-enzyme M (HSCH₂CH₂SO₃⁻), required for the methanogenic stage. It can thus inhibit the growth of methanogenic bacteria. Preliminary tests showed that a minimum dose of 1 M of BESA was required to sufficiently inhibit the activity of methanogenic bacteria in the present sludge. This dose was applied to the original sludge at 35°C to suppress the activity of the methanogenic bacteria.

5) Freezing and thawing—the sludge was frozen at −17°C for 24 h and then thawed for another 12 h in a water bath at 25°C (Hung et al., 1997). The freezing and thawing released considerable amounts of insoluble organic matter, particularly polysaccharides, to water as the ice front moved (Hung et al., 1996).

The original and pre-treated sludges were the substrates used in the hydrogen fermentation tests.

2.3. The inoculum

The inoculum was isolated from the collected wastewater sludge. The applied procedures include: (i) sterilizing at 121°C and 1.2 kgf cm⁻² (Huxley Autoclave, HL-360) for 30 min to inactivate the methanogenic bacteria (Lay, 2000); (ii) adding 100 mM of BESA to the sterilized sludge from step (i) for 24 h under anaerobic conditions; and (iii) isolating and purifying the incubated sludge in step (ii). Step (iii) include colonization of the incubated sludge on a gel-type reinforced clostridial medium for 72 h, three-time isolation of strains by removing and incubating colonies on the agar and the final selection of the strains by preliminary hydrogen production tests. The strains with the highest hydrogen yields were used as the inoculum in this study. These strains were analyzed at the following steps: DNA extraction, PCR amplification of 16S DNA gene, purification of PCR products, cycle sequencing, further purification and electrophoresis on the ABI Prism 377 DNA Sequencer. The sequence analysis of the 16S rDNA in the inoculum showed that it is a member of the clostridia family, Clostridium bifermentans.

2.4. Fermentation and testing

Substrate (45 ml; original or pre-treated) was mixed with 5 ml of inoculum suspension and was anaerobically incubated at 35°C in 125 ml serum bottles without stirring or further addition of nutrients. The bottles were capped with butyl rubber stoppers and wrapped in aluminum foil to prevent photolysis of the substrate (Chang et al., 1996). Gas and liquor samples were extracted at 8, 16, 24, 32, 40, 48, 72 and 96 h of fermentation. At each time and for each substrate (original or pre-treated), three serum bottles were arbitrarily selected and their average of hydrogen concentrations was reported. After measurements, the selected samples were abandoned to prevent the introduction of possible errors due to sampling, including gas leakage. Seven batches, including over 189 bottles (six batches × nine intervals × three bottles per measurement) of samples, were prepared.

A GC-TCD (Shimadzu, GC-8A), equipped with a stainless column packed with Porapack Q (50/80 mesh) at 70°C and a thermal-conductivity detector (TCD), measured the hydrogen and methane concentrations in the gas phase. The temperature of the injector and the detector of GC was maintained at 100°C. Nitrogen flowed at 20 ml min⁻¹ as the carrying gas. An integrator (HP3396 Series II) integrated the area under the peak of the effluent curve and quantified the gaseous concen-
<table>
<thead>
<tr>
<th>Author</th>
<th>Reactor type</th>
<th>Substrate</th>
<th>Seed</th>
<th>Maximum yield (g kg(^{-1}) DS)</th>
<th>Maximum yield (g kg(^{-1}) VSS)</th>
<th>Maximum yield (g kg(^{-1}) COD)</th>
<th>Maximum yield (g kg(^{-1}) substrate consumed)</th>
<th>Specific rate of hydrogen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tanisho and Ishiwata (1994)</td>
<td>Continuous reactor</td>
<td>Molasses</td>
<td>Enterobacter aeroenes</td>
<td>20.8(^*)</td>
<td>20.8(^a)</td>
<td>19.5</td>
<td>27.8 (based on sucrose)(^*)</td>
<td>4.13 g kg(^{-1}) sugar h(^{-1})</td>
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<td>Continuous reactor with urethane foam to support the flocs</td>
<td>Molasses</td>
<td>Enterobacter aeroenes</td>
<td>15.6(^*)</td>
<td>15.6(^a)</td>
<td>14.6</td>
<td>38.9 (based on sucrose)(^*)</td>
<td>1.3 g kg(^{-1}) sugar h(^{-1})</td>
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<tr>
<td>Kataoka et al. (1997)</td>
<td>Continuous reactor</td>
<td>Glucose and poly-peptone paper</td>
<td>Clostridium butylicum</td>
<td>24.44(^*)</td>
<td>24.44(^a)</td>
<td>23.0</td>
<td>NA</td>
<td>2.6 g H(_2) kg(^{-1}) glucose h(^{-1})</td>
</tr>
<tr>
<td>Sparling et al. (1997)</td>
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<td>Paper</td>
<td>Clostridium thermoscellum</td>
<td>0.50(^*)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>0.002 g kg(^{-1}) DS h(^{-1})</td>
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<td>Paper</td>
<td>Undefined consortium</td>
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<td>Lay et al. (1999)</td>
<td>Serum bottles</td>
<td>Organic municipal solid waste</td>
<td>Sludge of anaerobic digester</td>
<td>NA</td>
<td>14 (37°C)(^*)(^,b)</td>
<td>NA</td>
<td>NA</td>
<td>3.54 g kg(^{-1}) VSS h(^{-1}) (37°C)</td>
</tr>
<tr>
<td>Lin and Chang (1999)</td>
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<td>Glucose</td>
<td>Consortium from sewage digester</td>
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<td>19.0(^a)</td>
<td>17.8</td>
<td>23.3</td>
<td>–</td>
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<tr>
<td>Zhu et al. (1999)</td>
<td>Batch reactor with agar gel immobilization</td>
<td>Tofu wastewater</td>
<td>Immobilized Rhodobacter sphaeroides</td>
<td>19.3 (30°C)(^*)(^,b)</td>
<td>19.3 (30°C)(^*)(^,b)</td>
<td>NA</td>
<td>NA</td>
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<tr>
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<td>Glucose</td>
<td>Immobilized Rhodobacter sphaeroides</td>
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<td>29.8 (30°C)(^*)(^,b)</td>
<td>27.9 (30°C)(^*)(^,b)</td>
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<td>–</td>
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<td>Batch reactor</td>
<td>Centrate of boiling treated sludge</td>
<td>Consortiums in boiled treated sludge</td>
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<td>NA</td>
<td>1.42(^*)</td>
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<td>–</td>
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<td>Boiling treated sludge</td>
<td>Consortiums in boiled treated sludge</td>
<td>1.03</td>
<td>1.44(^*)</td>
<td>0.40</td>
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<td>Waste activated sludge starch</td>
<td>Consortiums in digested sludge</td>
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<tr>
<td>Lay (2000)</td>
<td>Serum bottles</td>
<td>Paper</td>
<td>Sludge from anaerobic digester</td>
<td>95.6</td>
<td>95.6(^a)</td>
<td>102 (37°C)(^*)(^,b)</td>
<td>NA</td>
<td>6.54 g kg(^{-1}) VSS h(^{-1})</td>
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<tr>
<td>Mizuno et al. (2000a)</td>
<td>Continuous reactor without nitrogen sparging</td>
<td>Glucose</td>
<td>Clostridium sp.</td>
<td>9.3(^*)</td>
<td>9.3(^a)</td>
<td>8.7</td>
<td>9.4(^*)</td>
<td>6.84 g kg(^{-1}) biomass h(^{-1})</td>
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<tr>
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<td>Glucose</td>
<td>Clostridium sp.</td>
<td>15.8(^*)</td>
<td>15.8(^a)</td>
<td>14.8</td>
<td>15.9(^*)</td>
<td>14.9 g kg(^{-1}) biomass h(^{-1})</td>
</tr>
<tr>
<td>Author</td>
<td>Reactor type</td>
<td>Substrate</td>
<td>Seed</td>
<td>Maximum yield (g kg&lt;sup&gt;-1&lt;/sup&gt; DS)</td>
<td>Maximum yield (g kg&lt;sup&gt;-1&lt;/sup&gt; VSS)</td>
<td>Maximum yield (g kg&lt;sup&gt;-1&lt;/sup&gt; COD)</td>
<td>Maximum yield (g kg&lt;sup&gt;-1&lt;/sup&gt; substrate consumed)</td>
<td>Specific rate of hydrogen</td>
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<tr>
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<td>Bean curd manufacturing waste</td>
<td>Anaerobic microflora</td>
<td>5.2</td>
<td>6.3</td>
<td>NA</td>
<td>28.22 (based on hexose)*</td>
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<tr>
<td>Bai et al. (2001)</td>
<td>Serum bottles</td>
<td>Peptone</td>
<td>Boiling treated sludge from UASB</td>
<td>1.98 (35 °C)&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>1.98 (35 °C)&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>0.69 (35 °C)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>NA</td>
<td>–</td>
</tr>
<tr>
<td>Lay (2001)</td>
<td>Serum bottles</td>
<td>Crystalline cellulose</td>
<td>Sludge from anaerobic digester</td>
<td>4.36&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.36&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.0</td>
<td>NA</td>
<td>1.5 g kg&lt;sup&gt;-1&lt;/sup&gt; VSS h&lt;sup&gt;-1&lt;/sup&gt;</td>
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<td>Liang et al. (2001)</td>
<td>Serum bottles</td>
<td>Peptone</td>
<td>UASB sludge inhibited by chloroform</td>
<td>2.96&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>1</td>
<td>NA</td>
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<td>UASB sludge inhibited by chloroform</td>
<td>9.92&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.92&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>Lin et al. (2001)</td>
<td>Continuous reactor</td>
<td>Alkaline-hydrolyzed waste activated sludge</td>
<td>Boiling treated sludge from sewage wastewater plant</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>1.7 (based on TCOD removed)*</td>
<td>0.05 g kg&lt;sup&gt;-1&lt;/sup&gt; TCOD h&lt;sup&gt;-1&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> All substrates are assumed volatile and ash-free.

<sup>b</sup> The hydrogen was assumed to be collected 1 atm.

<sup>*</sup> These values were reported in the original work.
trations. Repeated measurements showed that the determined hydrogen and methane contents included a maximum relative error of 15 and 10%, respectively. The hydrogen content in the anaerobic glove box was also measured and was subtracted from the hydrogen concentrations obtained in the serum bottles. The oxidative and reductive potential (ORP) and the pH value of the sludge were measured using an ORP-pH meter (HI 9017 Hanna). The \( \zeta \) potentials and the size distributions of the flocs of sludge were determined using a \( \zeta \) meter (Zetameter, Malvern) and a particle sizer (Mastersizer 2000, Malvern), respectively.

3. Results and discussion

3.1. Hydrolyzing organic matter

Fig. 1 plots the time evolution of SCOD for the fermented sludge. These data are normalized according to the chemical oxygen demand for the original sludge, COD\(_0\) (= 24,800 mg l\(^{-1}\)). Although some data are scattered, two points are notable. Firstly, the soluble COD of the original sludge is only 1.5% of COD\(_0\). All pre-treatments markedly increase this ratio to 4–5%, an enhancement of 150–250%. This finding is consistent with the findings of Lee and Mueller (2001). Secondly, the SCOD of the sludge samples increases to 8–16% after testing for 8 h.

3.2. Hydrogen production

Fig. 2 plots the quantity of produced hydrogen in the serum bottle obtained from GC-TCD tests. Contrary to the results obtained with a 66-h time lag by Cheng et al. (2000) for anaerobic fermentation, this work found a negligible time lag, which may be attributable to the fact that the inoculum was derived directly from the substrate sludge. The hydrogen concentration in the gas phase follows an increasing–decreasing curve, with a peak at 8–24 h, implying that a certain quantity of produced hydrogen has been in some way ‘consumed’. Cheng et al. (2000) reported a similar ‘hydrogen consumption’ phase during anaerobic fermentation.

Fig. 3 presents the specific hydrogen yield based on a gram of COD (mmol-H\(_2\)/g-COD\(_i\)), where COD\(_i\) is the COD of the substrate prior to testing, which is the inoculum + original sludge or + pre-treated sludge. Table 2 lists the COD\(_i\) values for all pre-treated samples prior to fermentation. In fact, all COD\(_i\)s are close to COD\(_0\), but with certain data fluctuation. A large data fluctuation is commonly noted for sludge tests since the collected sample is heterogeneous in nature.

As Fig. 4 shows, the specific hydrogen yield could reach 0.6 mmol g\(^{-1}\)-COD\(_i\) for the original
sludge, equivalent to 0.9 mmol g⁻¹-dried sludge in the system of interest. This value far exceeds that reported by Huang et al. (2000) (0.08 mmol-H₂/g-dried sludge). Meanwhile, freezing and thawing and sterilization markedly increase the specific hydrogen yield from ≈0.6 for the original sludge to 1.5–2.1 mmol-H₂/g-COD. Acidifying sludge did not significantly promote the production of bio-hydrogen. Furthermore, adding BESA and ultrasonication reduced the bio-hydrogen yield. The SCODs for all pre-treated sludge were higher than the original sludge (Fig. 1). Hence, not all organic matter released from the sludge is readily anaerobically fermented to hydrogen.

3.3. Methane production

Fig. 4 presents the amount of accumulated methane gas in the serum bottles with time. The amount of methane monotonically increases during fermentation with the magnitude of methane amount in the order, original > ultrasonically-treated or freeze/thawed > acidified or BESA-added > sterilized sludge. The amounts of methane produced after 96 h from the original (0.26 mmol-CH₄/g-dried sludge) and sonicated samples are 0.26 and 0.063 mmol-g-CH₄/g-dried sludge, respectively. These values are much lower than those obtained by Chu et al. (2002b): 1.6 and 5 mg-CH₄/g-dried sludge, respectively, when methanogenic bacteria were externally seeded at the start of the digestion test. The pretreatments effectively deactivated most methanogenic bacteria in the substrate in this study. The hydrolyzed organic matter (increased COD in Fig. 1) was not effectively converted to methane gas by the inoculum.

BESA effectively suppressed methane production to ≈50% of the original sludge. However, hydrogen production was not enhanced. This methanogenic inhibitor, at a high concentration of 1 M, might disrupt the fermentation pathway by Clostridium sp., or might not really be able to block out all hydrogen-consuming pathways taken by other bacteria, including sulfur-reducing bacteria.
3.4. Species-dependent fermentation efficiency

Figs. 5 and 6 plot pH and ORP for the suspension during the fermentation test, respectively. For all samples, the pH value slowly approaches 5.5 and ORP approaches about 300 mV. The ζ potential and the floc size of the fermented samples (data not shown) were also measured. All samples, after 24 h of fermentation, exhibited a ζ potential between −13 and −17 mV and a size between 60 and 70 μm, except in the freeze/thawed case. Although the sludge samples yielded different hydrogen or methane productivities, they were converted to entities of similar size and surface charge. Meanwhile, the suspension approached a similarly reductive environment. The changes in environmental factors were insignificant. Jones and Woods (1986) suggested that because of the accumulation of fatty acid in suspension, the fall in pH during fermentation prevented the production of hydrogen from biomass. This suggestion does not apply in this study because of the strong buffering capacity of the sludge. Some of the consumed hydrogen was transformed into methane. However, the ‘consumption’ of formed hydrogen (Fig. 3) and the production of methane (Fig. 4) are not correlated. For instance, the peak hydrogen productivity for freeze/thawed sludge, occurring after 16 h of fermentation, was ≈ 4.4 mg/g-COD. This amount of hydrogen was mostly consumed at hour 40, meanwhile, the corresponding methane production was only 0.28 mg/g-COD. The methane-producing pathway can therefore partially account for the hydrogen-consuming phase.

The various hydrogen yields following various pre-treatment processes imply that not all the released COD are ready for hydrogen formation. The species released from the sludge should correspond to the observed differences in bio-hydrogen yields since the changes in all environmental factors were minimal. Hung et al. (1996) showed that freezing and thawing released considerable amounts of cellulose but retained most lipids or protein on the solids phase. Different pre-treatments may thus ‘fractionate’ the components in the solids and the aqueous phase, thereby modifying the hydrogen productivity. For example, the increase in the amount of cellulose in suspension after freezing and thawing may correspond to the increase in hydrogen yield. However, ultrasonication may release sufficiently large amounts of undesired compounds that suppress the fermentation. Releasing a fraction of COD to prepare for hydrogen production is essential to facilitate efficient fermentation. Further works are now in progress to elucidate this point.

4. Conclusions

This work is the first systematic study of the use of C. bifermentans to produce hydrogen from
wastewater sludge. The hydrogen yields reported in the literature ranged from 4.6 to 15 mmol-H₂/g-dried solids (DS) for fermenting glucose-containing solution (Zhu et al., 1999; Lin and Chang, 1999; Mizuno et al., 2000a; Liang et al., 2001) or 1–1.5 mmol-H₂/g-DS from the protein-containing solution (Bai et al., 2001; Liang et al., 2001). Meanwhile, the hydrogen yield by anaerobically fermenting wastewater sludge is rather low at ≈ 0.08 mmol-H₂/g-dried solids (Huang et al., 2000). We demonstrated a hydrogen yield for the original wastewater sludge up to 0.9 mmol-H₂/g-dried solids, close to that fermented from protein-containing wastewater. The effects of five different pre-treatments, ultrasonication, acidification, sterilization, freezing and thawing, and adding methanogenic inhibitor, on the hydrogen yield were investigated. Freezing and thawing and sterilization markedly increased the specific hydrogen yield to 1.5–2.1 mmol-H₂/g-COD. This yield exceeds that obtained from thermally boiled sludge by Cheng et al. (2000) (0.7 mmol-H₂/g-COD). However, adding methanogenic inhibitor or performing ultrasonication reduced bio-hydrogen yield.

The hydrogen accumulation curve presents increasing–decreasing characteristics. After the peak is reached, a particular amount of methane is formed. No clear correlation exists between the reduction in the amount of produced hydrogen and the amount of methane converted from produced hydrogen, implying that only some hydrogen was converted into methane. Pre-treatments effectively released insoluble organic matter into water. Furthermore, the soluble chemical oxygen demand (SCOD) increased and approached a plateau after the first 8 h of fermentation of all samples. Examining the environmental factors revealed that changes in pH, ζ potential or floc size did not relate to the observed consumption of hydrogen. Thus, not all released COD is ready for hydrogen formation.

References


