Biohydrogen production from industrial wastewaters
Iván Moreno-Andrade, Gloria Moreno, Gopalakrishnan Kumar and Germán Buitrón

ABSTRACT

The feasibility of producing hydrogen from various industrial wastes, such as vinasses (sugar and tequila industries), and raw and physicochemical-treated wastewater from the plastic industry and toilet aircraft wastewater, was evaluated. The results showed that the tequila vinasses presented the maximum hydrogen generation potential, followed by the raw plastic industry wastewater, aircraft wastewater, and physicochemical-treated wastewater from the plastic industry and sugar vinasses, respectively. The hydrogen production from the aircraft wastewater was increased by the adaptation of the microorganisms in the anaerobic sequencing batch reactor.

Key words | aircraft wastewater, biohydrogen, plastic industry, sequencing batch reactor, vinasses

INTRODUCTION

The demand for energy is increasing more than ever before due to overusage by the modern world, which depends totally on high-level technologies. Contrastingly, the fossil fuel reservoirs that are the major source of power are diminishing gradually due to their non-renewable nature. Also, due to the carbonaceous nature, the fossil fuels have created a lot of environmental issues including global warming. Thus, finding an alternative or renewable energy sources that are clean has been paid more attention in recent years (Brennen & Owende 2010). In this spotlight, hydrogen has been considered and demonstrated as a future fuel by various researchers around the globe (Wang & Wan 2009a). Hydrogen (H₂) is a clean energy that does not produce greenhouse gases and has a high-energy content by weight in comparison with other fuels: 120.9 kJ/g of energy versus 48.3 and 44 kJ/g for gasoline and natural gas, respectively. Microorganisms can convert biomass into H₂ by a biological fermentation of organic waste compounds, which include industrial wastewater, lignocellulose waste and municipal waste (Lin et al. 2012).

Hydrogen production potential (HPP) from various feedstocks has been demonstrated (Wang et al. 2009a; Ramos et al. 2012; Buitrón & Carvajal 2010). Among these substrates, many industrial wastewaters such as pharmaceutical, textile and food processing industry wastewaters have showed inhibition to the hydrogen production, due to the presence of toxic compounds such as phenols, benzenes, halogenated aliphatics, N-substituted aromatics (Lay et al. 2012; Chu et al. 2013; Krishna et al. 2013). For this reason, it is necessary to evaluate the HPP from different industrial feedstocks that could contain inhibitory compounds in order to determine the potential use for H₂ production by dark fermentation. It has been reported that the use of anaerobic sequencing batch reactors (AnSBRs) has the potential to treat many chemical and toxic wastes and is proved to be an effective way to produce hydrogen (Buitrón & Carvajal 2010; Krishna et al. 2013).

The objective of this study was to evaluate the potential of different industrial feedstocks to produce biohydrogen. The outcomes of this research could be useful to develop a sustainable hydrogen production technology using industrial effluents commonly considered as waste.

MATERIALS AND METHODS

Industrial feedstock collection and seed inoculum

Different industrial wastewaters were used as a substrate to evaluate the potential for hydrogen production and were collected from the origin of the industries. The feedstocks used were (1) sugarcane vinasses, (2) tequila vinasses, (3) raw wastewater from a plastic industry, (4) physicochemically treated wastewater from a plastic industry, and (5) toilet wastewater generated in aircrafts. The seed inoculum was anaerobic sludge pre-treated by thermal shock (103–106 °C).
over 24 h) to inhibit the activity of methanogens, and to select hydrolytic–acidogenic microorganisms that are responsible for the production of hydrogen.

**Experimental strategy**

The experimental strategy of this study was divided into two parts: (1) evaluation of the hydrogen production potential of the feedstocks; and (2) operation of a sequencing batch reactor with aircraft toilet wastewater.

**Hydrogen production potential**

Different initial concentrations of chemical oxygen demand (COD) of the feedstocks were tested to evaluate the HPP: 1,000, 2,000 and 3,000 mgCOD/L (except for the vinasses where the COD tested was 3,000 mgCOD/L). HPP was evaluated in serum bottles of 120 mL with a working volume of 80 mL. Deoxygenated water was added to fill the total volume. pH values in the experiments were adjusted to 5.5 using an organic buffer MES (2-(N-morpholino)ethanesulfonic acid) with a buffer capacity of 10 mM. Incubation was at constant temperature of 36°C, and the content was mixed using an orbital shaker at 130 rpm. All experiments were run in triplicate, and the standard deviation values are reported.

**Operation of a sequencing batch reactor with aircraft toilet wastewater**

After the evaluation of HPP experiments, the treatability of aircraft toilet wastewater was evaluated in a sequencing batch reactor in order to determine the changes in hydrogen production by continuous acclimatization of the biomass in several cycles. An AnSBR of a total volume of 1.5 L and an exchange volume of 50% was employed. The reactor was completely mixed at 150 rpm. Temperature was controlled at 35 ± 1°C via a water bath. The reaction phase was 48 h with a settling time of 30 min. The same inoculum used in the HPP test was investigated.

**Gas and liquid analysis**

The production of the biogas was measured applying the methodology described by Shelton & Tiedje (1984). Biogas was estimated with the pressure, temperature and volume that prevailed during the test. Endogenous biogas production was evaluated by tests with no substrate addition for the inoculum, and was subtracted in the biogas calculation. The hydrogen percentage in the biogas was determined using gas chromatography (Agilent 6890/TCD, column Supelco-Carboxen 1010 Plot).

The calculations for the kinetic analysis were performed based on the cumulative H2 production obtained. The modified Gompertz equation (Equation (1)) was used for data analysis (Ramos et al. 2012).

\[
H(t) = H_{\text{max}} \exp \left[ -\exp \left( \frac{2.71828 \cdot R_{\text{max}} (\lambda - t)}{H_{\text{max}}} + 1 \right) \right]
\]

where \( H(t) \) (mL/L-reactor) is the total amount of hydrogen produced at culture time \( t \) (h); \( H_{\text{max}} \) (mL/L-reactor) is the maximal amount of hydrogen produced. \( R_{\text{max}} \) (mL/L-reactor·h) is the maximum hydrogen production rate; \( \lambda \) (h) is the lag time before the exponential hydrogen production.

Analytical techniques applied also include total and volatile suspended solids, which were determined according to Standard Methods (APHA 2005). The COD analysis was measured applying the Hach Small Tube COD Cuvette Test, according to the manufacturer instructions. The phenol content was measured as total phenols using the colorimetric technique of 4-aminoantipyrine according to Standard Methods (APHA 2005). Volatile fatty acids were determined by gas chromatography (flame ionization detector) according to an earlier study (Hernández-Mendoza & Buitrón 2014).

**RESULTS AND DISCUSSION**

**Characterization of the industrial feedstocks**

The characterization of the substrates used in this study is shown in Table 1. It can be clearly seen that almost all feedstocks have high COD values, which is favorable for hydrogen production. The COD values range from 191.2 to 99.6 mgCOD/L for plastic wastewater and sugar vinasses, respectively. The total phenols content (mg/L) also varies significantly, with values ranging from 104.9 mg/L for raw plastic wastewater to 14.0 mg/L for tequila vinasses. The N-NH3 (mg/L) values are relatively low, ranging from 45 to 700 mg/L. The BOD5 values (g/L) are also provided, with values ranging from 5 to 25.1 g/L for the different feedstocks.

<table>
<thead>
<tr>
<th>Feedstock</th>
<th>Color</th>
<th>COD (g/L)</th>
<th>Total phenols content (mg/L)</th>
<th>N-NH3 (mg/L)</th>
<th>BOD5 (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sugar vinasses</td>
<td>Brown</td>
<td>99.6</td>
<td>46.2</td>
<td>700</td>
<td>25.1</td>
</tr>
<tr>
<td>Tequila vinasses</td>
<td>Brown</td>
<td>40.0</td>
<td>14.0</td>
<td>300</td>
<td>32.5</td>
</tr>
<tr>
<td>Raw plastic wastewater</td>
<td>Dark blue</td>
<td>191.2</td>
<td>104.9</td>
<td>50</td>
<td>55</td>
</tr>
<tr>
<td>Physicochemical-treated plastic wastewater</td>
<td>Blue</td>
<td>7.3</td>
<td>26.0</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>Aircraft’s toilet wastewater</td>
<td>Greenish blue</td>
<td>5.4</td>
<td>&gt;0.1</td>
<td>125</td>
<td>5.4</td>
</tr>
</tbody>
</table>

\[ \text{BOD}_5 \]: 5-day biochemical oxygen demand. Note that \( \text{BOD}_5 \) was not evaluated for plastic wastewaters.

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the wastes contain phenols, which was shown as an inhibitory compound for microorganism growth (Buitrón et al. 2005). The content of total phenols ranged from 14 to 105 mg/L and the highest was observed in raw plastic wastewater. COD of the feedstocks ranged from 5 to 191 gCOD/L. Peak COD was also seen in raw plastic industry wastewater, and high alkalinity of 41.6 g CaCO₃/L was found. High sulfate concentration was found for sugar (6.5 g/L) and tequila (0.8 g/L) vinasses. High concentration of sulfate also proved to be inhibitory in other studies (Guo et al. 2010). Some previous reports are available for the production of H₂ and CH₄ from tequila vinasses (Espinoza-Escalante et al. 2009; Buitrón & Carvajal 2010). However, no earlier reports used plastic industry wastewaters or aircraft toilet wastewater.

**Hydrogen production potential**

The production performances of the employed substrates are shown in Figures 1–3. Dots in the figures show the experimental data obtained and lines represent the fit to the Gompertz model.

Tequila vinasses showed the maximum H₂ production among the feedstocks evaluated. For this effluent, the maximum amount of hydrogen production (Hₘₐₓ) was 721 mL H₂/Lₜₐₐₐₚₐₒ₉₉ and the specific hydrogen production rate (SHPR) was 7.1 mL H₂/(gCOD-h). Sugar vinasses showed a low capacity to generate hydrogen since the Hₘₐₓ was 113 mL H₂/Lₜₐₐₐₚₐₒ₉₉ and the SHPR was as much as 5.0 mL H₂/(gCOD-d) (Figure 1). Acetic acid was analyzed as the principal intermediate compound in the metabolism for both cases. The results obtained agree with those obtained by Buitrón & Carvajal (2010), where a SHPR between 3.7 and 14.3 mL H₂/gCOD/h was obtained in an AnSBR operated at 35°C and a hydraulic residence time of 24 h. The case of sugar cane wastewater, Ueno et al. (1996) obtained values of SHPR from 2.9 to 3.2 mL H₂/gCOD/h. The lower hydrogen production from vinasses compared with easy-to-degrade wastewater (e.g., municipal wastewater) can be related to the presence of inhibitory compounds in this wastewater (e.g., phenol and sulfate). It has been observed that toxic wastewater containing cellobiose and phenol can be utilized for hydrogen production applying pure cultures, e.g., Clostridium butyricum (Lee et al. 2011). However, Lee et al. (2011) found that the hydrogen production rate decreases as the phenol concentration increases.
Hydrogen production from raw and physicochemical-treated wastewater from the plastic industry is shown in Figures 2(a) and 2(b), respectively. The maximal peak of hydrogen production was observed at 75 and 145 h for the raw and physicochemical-treated plastic wastewater, respectively. This difference is clearly related to the lag time (from 1 to 2 h, and from 61 to 66 h for raw and physicochemical-treated wastewater, respectively). The maximal hydrogen production was obtained at 3 gCOD/L (281 and 109 mL H2/Lreactor for raw and physicochemical-treated wastewater, respectively).

The physicochemical-treated wastewater had a higher phenol/COD ratio (3.56 gphenol/gCOD) than the raw wastewater (0.55 gphenol/gCOD). It has been shown that acclimated microorganisms can degrade phenol efficiently at phenol/COD ratio of 0.8 or lower (Bajaj et al. 2008). In the case of the treated wastewater, the COD was reduced by the application of a polymeric substance (in a physicochemical process) removing 96% of the total COD, and only 75% of the phenol, increasing the phenol/COD ratio. This increase in phenol can be related to the diminution of hydrogen production; however, other compounds not revealed in the characterization can also affect the H2 production. Tai et al. (2010) showed that the increase in phenol content in synthetic wastewater generated a decrease in the hydrogen production, reporting a complete inhibition of cell activity at phenol concentration higher than 1 g/L.

The aircraft toilet wastewater is characterized by a high COD, undiluted organic matter and toilet paper. To our knowledge, the use of wastewater from aircraft toilet as feedstock has never been reported for H2 production. This feedstock might contain formaldehyde, glyoxal, glutaraldehyde, quaternary ammonium, alkyl phenols and glycols and other chemicals, which can inhibit the growth and activity of microorganisms (Wegner et al. 1978). The results of the HPP for different initial COD concentrations are shown in Figure 3. It is possible to observe that H2 production increased as the initial COD increased. However, after 2,000 mgCOD/L the H2 production dropped because of the inhibition to microorganisms, produced by the compounds present in aircraft toilet wastewater. The maximal H2 production without inhibition was 285 mL H2/Lreactor. The maximal hydrogen percentage (17%) in biogas was also observed at the concentration of 2,000 mgCOD/L.

**Kinetic and energy production analysis**

The importance of the kinetics of hydrogen production has been explained in many studies (Wang & Wan 2009b; Sivagurunathan et al. 2014). Since kinetic parameters are useful for the scale-up of the process, the main parameters such as lag phase time, $H_{\text{max}}$ and $R_{\text{max}}$ values were determined by fitting the production data with the Gompertz model (Table 2). The lag phase time ($\lambda$) values ranged from 1 to 66 h according to the type of waste feedstock used. Short lag phase time (1 h) was noted with the sugar vinasses, whereas longer startup time of 66 h was observed with the plastic industry wastewater feedstock; this is mainly due to the high toxic nature of the latter. For the $R_{\text{max}}$, the higher values were obtained by the tequila vinasses, while the lowest values were obtained with raw plastic and toilet aircraft wastewater (0.3 and 0.4 mL H2/(Lreactor·h), respectively, at 1 g of initial COD). Figure S1 in the supplementary material (available online at http://www.iwaponline.com/wst/071/471.pdf) shows the comparison of all the wastewaters applying 3,000 mgCOD/L. It is possible to observe

<table>
<thead>
<tr>
<th>Feedstocks</th>
<th>COD Initial (g/L)</th>
<th>Lag phase time (h)</th>
<th>$H_{\text{max}}$ (mL H2/Lreactor)</th>
<th>$R_{\text{max}}$ (mL H2/(Lreactor·h))</th>
<th>Energy production yield (KJ/gCODinitial)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sugar vinasses</td>
<td>3</td>
<td>1</td>
<td>113</td>
<td>15.0</td>
<td>0.48</td>
</tr>
<tr>
<td>Tequila vinasses</td>
<td>3</td>
<td>16</td>
<td>721</td>
<td>21.3</td>
<td>3.07</td>
</tr>
<tr>
<td>Raw plastic wastewater</td>
<td>1</td>
<td>1</td>
<td>13</td>
<td>0.3</td>
<td>0.17</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>4</td>
<td>58</td>
<td>2.8</td>
<td>0.37</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>12</td>
<td>281</td>
<td>8.0</td>
<td>1.20</td>
</tr>
<tr>
<td>Physicochemical-treated plastic wastewater</td>
<td>1</td>
<td>66</td>
<td>90</td>
<td>5.5</td>
<td>1.15</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>66</td>
<td>98</td>
<td>3.4</td>
<td>0.63</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>61</td>
<td>109</td>
<td>2.5</td>
<td>0.46</td>
</tr>
<tr>
<td>Aircraft toilet wastewater</td>
<td>1</td>
<td>14</td>
<td>45</td>
<td>0.4</td>
<td>0.57</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>16</td>
<td>285</td>
<td>3.9</td>
<td>1.82</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>12</td>
<td>230</td>
<td>4.0</td>
<td>0.98</td>
</tr>
</tbody>
</table>
that for a same initial concentration all the wastewaters tested reach the $H_{\text{max}}$ at 150 h or less. The higher $R_{\text{max}}$ and $H_{\text{max}}$ were observed for tequila vinasses. It is interesting to note that the sugar vinasses reached very quickly the $H_{\text{max}}$ (at 18 h); however, this is the lower value of $H_{\text{max}}$ for all the feedstocks tested. This demonstrates that the maximal hydrogen production is not correlated to the minimal lag phase or the higher $R_{\text{max}}$.

The details of the energy production values for various feedstocks tested were calculated taking into account the maximal amount of hydrogen produced and the heating value parameter of 285.8 J/mmol H$_2$ and are reported in Table 2. The higher values were attained from tequila vinasses as 3.07 kJ/gCOD$_{\text{initial}}$. An interesting point is that the effluents of this fermentation could be used to produce biohydrogen by photo-fermentation (due to the volatile fatty acids produced) or methane by methanogenic processes to increase the total bioenergy production yield of the process.

**AnSBR operation of aircraft toilet wastewater**

As the HPP experiments are designed considering unacclimated biomass and the time restricted the study to only one batch, we decided to test the aircraft toilet wastewater applying an AnSBR reactor in order to acclimate the biomass to the H$_2$ production and to evaluate if the H$_2$ production potential can increase after several operation cycles. The aircraft wastewater was selected, as an example of the tested feedstocks, for its evaluation in a laboratory-scale pilot reactor because previous studies have demonstrated that acclimation of microorganisms can increase the biodegradation in continuous and discontinuous processes (Moreno-Andrade et al. 2014). The AnSBR was operated for 19 degradation cycles of 48 h each (38 d of operation). Figure 4 shows the hydrogen production performance of the AnSBR. It is possible to observe that after 14 cycles of operation (28 d), the volume of biohydrogen increased from 120 to 430 mL/(L$_{\text{reactor}}$·d). Also, hydrogen content in produced gas increased from 20 to 42%. That evidenced that the long-term operation of a biomass in a reactor can increase the capability of the microorganisms to produce H$_2$. A possible reason for this is the acclimation of the microorganisms to the toxic substances after a long exposure period. This kind of observation has been demonstrated previously for 4-chlorophenol degradation (Moreno-Andrade & Buitrón 2004). Acclimation also revealed that the values obtained in the HPP test could be significantly increased via continuous mode of operation for biohydrogen production.

**CONCLUSIONS**

This study demonstrated the possibilities of the hydrogen production from various industrial feedstocks containing inhibitory compounds. The tequila vinasses presented the maximum hydrogen production potential, followed by the plastic industry wastewater, aircraft wastewater, physico-chemical-treated wastewater from the plastic industry and sugar vinasses, respectively. The hydrogen production from the aircraft wastewater was increased by the adaptation of the microorganisms in the AnSBR.

**ACKNOWLEDGEMENTS**

The authors gratefully acknowledge the financial support of PAPIIT project IN103315 and Bita Project (Marie Curie) PIRSES-GA-2011-295170. Jaime Pérez Trevilla is acknowledged for his technical assistance. Andres Martinez, Alfredo Montes, Geovana Cabello and Adan Silva are acknowledged for their participation in the test experiments.

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First received 10 June 2014; accepted in revised form 10 November 2014. Available online 22 November 2014