Research Article

Application of the Single-Stage Process of Dark Fermentation and Microbial Electrolysis to Improve Hydrogen Productivity from Water Hyacinth

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Received 25 April 2023; Revised 17 October 2023; Accepted 18 October 2023; Published 16 November 2023

Academic Editor: Thangjam Ibomcha Singh

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The production of hydrogen (H2) from water hyacinth (WH) can contribute to reducing both the negative impact of WH on ecosystems and dependence on fossil fuels. In this study, the combination of dark fermentation (DF) and microbial electrolysis cell (MEC) in a single reactor, namely, sDFMEC, was investigated to improve the H2 productivity of WH. Furthermore, the intermittently applied voltage (I-Eapp) scheme and various methane (CH4) inhibition methods, including air exposure, heat treatment, and chloroform (CHCl3) addition, were applied for performance enhancement purposes. The findings indicated that with a sufficient duty time of external energy input (less than 1 hour), the intermittent mode can enhance the performance of WH-fed sDFMEC but does not significantly inhibit CH4 formation. While air exposure and heat treatment damaged both methanogens and exoelectrogens, lowering sDFMEC performance, additional CHCl3 showed the best selective and long-term inhibition on methanogens (over 350 operation hours without further addition). Overall, the combination of the I-Eapp scheme and CHCl3 applied in WH-fed sDFMEC achieved a yield of 670 ± 15.2 mL H2/g VS, around 160% higher than the normal condition.

1. Introduction

In tropical and subtropical areas, water hyacinth (WH) is treated as the most noxious weed that damages agricultural and aquatic ecosystems [1, 2], because its vigorous developmental characteristics can rapidly deplete oxygen and nutrient contents in water bodies, leading to the death of other plants and animals [1]. Fortunately, WH contains high carbohydrates, a favorable substrate for obtaining bioenergy from biological processes. Therefore, research on WH-derived biofuels, especially hydrogen (H2) [3–6], is receiving much attention. Additionally, H2 has been proven to be an excellent substitute for fossil fuels that has many advantages over other alternative energy sources [7, 8]. The reasons for this are that it is clean (the product when burning H2 is only water vapors, which obviously has no negative effects on the environment), has a high mass-specific energy (around 120 kJ/g, which is 2.7 times higher than gasoline), and can be easily used in fuel cells to generate electricity [9, 10], etc. Therefore, H2 produced from WH can assist in alleviating the negative impacts of WH while also contributing to reducing reliance on fossil fuels, ushering in a new era of sustainable energy.

Various biological H2 production methods, such as biophotolysis, dark fermentation (DF), photofermentation (PF), and microbial electrolysis cell (MEC), have been developed so far [7]. Among them, DF and MEC have received a lot of research attention because they can operate regardless
of weather conditions [11, 12]. In order to boost the H₂ productivity of WH, the combination of dark fermentation and microbial electrolysis cell in a two-stage process (i.e., DF-MEC) was investigated and showed a positive effect that can boost H₂ yield from WH by about nine times higher than that of the DF alone process [5, 6]. However, to ensure the effective operation of the DF-MEC process, many auxiliary processes (e.g., centrifugation, dilution, and pH adjustment) must be applied, thereby making the whole process more complicated [5]. To overcome the bottlenecks of the two-stage process, a single-stage DF and MEC, namely, sDFMEC, has recently been proposed [13–16]. With sDFMEC, both the DF and MEC processes can maintain their advantages in a single reactor, providing the maximal yield of 12 mol-H₂/mol-glucose from a variety of organic sources, while significantly reducing expenditures when compared to two-stage systems [14]. Indeed, an sDFMEC system permits coproduced soluble metabolite products (SMPs) from the fermentation of WH by fermenting bacteria to be simultaneously oxidized by exoelectrogens at the anode during the MEC stage. This concurrent process minimizes the buildup of volatile fatty acids and hence maintains the system’s pH balance [14, 16]. This self-supporting mechanism, therefore, makes the sDFMEC process more efficient, while the design structure is much simpler than the DF-MEC process.

However, studies on sDFMEC are still limited as it has only been studied on marine biomass substrates, such as macroalgae [14, 17], with no studies on lignocellulosic biomass. Besides, various issues of sDFMEC, such as poor purity of produced H₂ due to methane (CH₄) cogeneration and ineffective energy recovery, have not been properly overcome [11, 13]. Specifically, the coexistence of unwanted microorganisms can considerably reduce the efficiency of sDFMEC [14–16]. For instance, electrotrophic methanogens compete directly with the hydrogen evolution reaction (the main reaction for H₂ production at the cathode of sDFMEC), which reduces protons and electrons for CH₄ formation [18], as shown in Eq. (1). Besides, CH₃ is also generated through the activity of the hydrogenotrophic and the acetoclastic methanogens (Eq. (2) and Eq. (3), respectively), which directly and indirectly reduce H₂ production yield through consumption of H₂ and the competition of substrates (i.e., acetate) used in H₂ production [18, 19]. More seriously, the CH₄ formation also causes the purity of H₂ to be contaminated, which makes the downstream more complicated. Additionally, the activity of homoacetogens can result in an H₂-recycling or H₂-acetate loop, in which H₂ is consumed and acetate is rebuilt, as illustrated in Eq. (4). This loop increases the operating duration and external energy input, thus reducing the H₂ recovery of sDFMEC [20, 21]. Therefore, inhibiting the growth of undesirable methanogens is significant to maximize the H₂ productivity of sDFMEC.

\[
\begin{align*}
\text{HCO}_3^- + 9H^+ + 8e^{-}_{\text{electrotrophic methanogens}} & \rightarrow CH_4 + 3H_2O, \\
CO_2 + 4H_2 & \rightarrow CH_4 + 2H_2O,
\end{align*}
\]

(1) (2)

Up to now, there are many proposed methods for MEC, including air exposure, shorter operating cycles, additional chemical inhibitors (e.g., 2-bromoethane sulfonate, CHCl₃, and acetylene), low temperature (<10°C), heat treatment, using pure cultures, using ultraviolet irradiation, and using a gas-permeable membrane combined with a vacuum [13, 22]. Among them, applying a high voltage to shorter operating cycles can be considered a convenient, economical, and eco-friendly strategy [11]. Accordingly, beyond minimizing the formation of CH₄, high applied voltage (E_app) can promote the exoelectrogens’ growth [23, 24] and facilitate the transfer and reduction of protons, thereby preventing local acidification at the anode and improving the efficiency of sDFMEC [25]. However, the high applied voltage adversely affected the process’s energy efficiency due to increased energy consumption [5].

Fortunately, some recent studies indicated that such energy input in MEC operation could be minimized with the intermittent power input (I-E_app) mode instead of the traditional continuous mode (C-E_app) [26, 27]. Accordingly, with the applied voltage switched on and off for a certain duty length, it was observed that the energy efficiency improved more than that of the C-E_app mode in MEC operation [27]. Since MEC is involved in the functioning of sDFMEC, the I-E_app model may improve the performance of WH-fed sDFMEC. However, to clarify this hypothesis, the operating parameters in such an intermittent mode (e.g., duty time) need to be explored.

From the above background, this study is the first to investigate the efficacy of the sDFMEC for H₂ production from lignocellulosic biomass, specifically WH. Additionally, to improve the performance of WH-fed sDFMEC, intermittent external applied voltage with various duty lengths was investigated and expected to minimize CH₄ formation in the reactor. Besides, some other CH₄ inhibition methods are also examined for comparison and support purposes, including air exposure, heat treatment, and the use of CHCl₃. The findings obtained in this work can provide helpful insights into enhancing the H₂ productivity of other biomass sources, hence contributing to a reduction in reliance on gradually depleting fossil fuels.

2. Materials and Methods

2.1. Materials. The WH (Eichhornia crassipes) harvested from the Saigon River (located in Ho Chi Minh City, Vietnam) was used as the model substrate. After collection and removal of dirt with tap water, the samples were cut to roughly 3 cm, washed with deionized (DI) water, and dried at 105°C for 48 h. The dried samples were then milled in a commercial grinder, sieved to a size of 0.5–1.0 mm, and kept in sealed containers at 5°C for future usage. The properties of the WH sample are presented in Table 1. Before being fed to sDFMEC, the WH sample was pretreated with the
ultrasonic-assisted alkaline method for 60 minutes (on- and off-pulse times are 50 seconds and 10 seconds, respectively) in a 0.25 M sodium hydroxide solution with an ultrasonic processor (VCX 750, Sonics & Materials Inc., USA). All other chemicals were purchased from Sigma-Aldrich. DI water was utilized throughout the experiments.

2.2. sDFMEC Setup and Operation. The schematic of WH-fed sDFMEC with intermittent energy input and complete inhibition of undesirable microorganisms is illustrated in Figure 1. sDFMEC experiments were carried out in two single-chamber membrane-free reactors, which were constructed as described in the previous work [6]. Briefly, the anode and cathode were made of carbon cloth (7 × 10 cm, without wet proofing, E-Tek, USA) and stainless-steel mesh (7 × 16 cm, SUS304, 60 mesh, Nguyen Muon Co. Ltd., Vietnam), respectively. Additionally, the carbon cloth was used as received without further pretreatment, while the stainless-steel mesh was alternately sonicated in ethanol and DI water and then dried at 80 °C for 12 hours before use. The exoelectrogen biofilm on the anodes was established in the MECs that operated with DF effluent from WH (consisting of around 1.2 g-SMPs/L) for over three months [5, 6]. Both electrodes were circled and connected to an external circuit by SUS304 wires (0.8 mm in diameter). A 1000 mL gas sampling bag (Tedlar®, Sigma-Aldrich, USA) was connected to the top of each sDFMEC reactor for collecting the biogas produced. Before each batch experiment, each reactor was filled with 30 mL of pretreated-WH suspension (equivalent to 5 g-TS of WH per L), 30 mL of heat-treated (100°C, 30 minutes) anaerobic activated sludge (15.3 g-VSS/L, pH 6.8), 150 mL of PBS (100 mM, pH 7.0), chemical inhibitors (if any, depending on the inhibition methods, Section 2.3), and a given amount of DI-water to reach a working volume of 300 mL. The reactor was then aerated with pure N₂ gas for at least 20 minutes to ensure an anaerobic environment. An operating temperature of 35°C and a stirring speed of 150 rpm were kept constant during the experiment. The VSP-300 multichannel potentiostat (Bio-Logic, France) was used to apply voltage to both sDFMEC reactors in either continuous (C-E app) or intermittent (I-E app) mode, with the EC-Lab program (ver. 11, Bio-Logic) controlling and recording data.

2.3. Methanogens Inhibition Experiments. To prevent the activity of methanogens in sDFMEC, some physical (including air exposure and heat treatment) and chemical (i.e., CHCl₃) inhibition methods were examined. In the air exposure method, the anodes were exposed to natural air at room temperature (31 ± 1°C) for 30 minutes. The second is the heat-treated method, where the anodes were pretreated by heating in a 50 mM phosphate-buffered saline solution (PBS) at 100°C for 30 minutes. The remaining method used CHCl₃ as a chemical methanogen inhibitor, with a dosage of 20 ppm (v/v). These methods were applied at the start of the first cycle. Following that, the sDFMEC reactors continued to operate for multiple serial batch cycles without any additional inhibition to evaluate their long-term effectiveness. To simplify the experimental process, the continuous applied voltage mode (i.e., C-E app) was established for all of these inhibition methods. After determining the most effective inhibition method, it was further combined with the I-E app mode to investigate the overall performance of sDFMEC under such enhancement methods.

2.4. Analysis and Calculations. The produced biogas volume was determined by a volumetric displacement approach [14]. The contents of H₂ and CH₄ in the gas samples were measured by a gas chromatography system (7890A, Agilent, USA) with an HP PLOTQ capillary column, a thermal conductivity detector, and argon as carrier gas [6]. Total chemical oxygen demand (TCOD) and soluble chemical oxygen demand (SCOD) were analyzed using the Mn (III) method with a Hach® DR portable spectrophotometer (2700™, USA). H₂ production yield (YH₂, mL/g-VS) and rate (HPR, mL/L/h) were calculated following Eq. (5) and Eq. (6), respectively [15, 28]. The energy recovery based on electrical energy input (ηE, %) and the overall energy recovery based on both electrical and substrate energy input (ηE+iS, %) were determined according to Eq. (7) and Eq. (8), respectively [14]. The COD mass balance was computed based on instructions in [29], which is the ratio of the total COD of H₂ and CH₄ produced to the TCOD removed.

\[
Y_{H2} = \frac{1000 \times V_{H2}}{m_S \times C_{VS}}, \quad (5)
\]

\[
HPR = \frac{1000 \times V_{H2}}{V_R \times t}, \quad (6)
\]

\[
\eta_E = \frac{PV_{H2}}{RT} \times \frac{\Delta H_{H2}}{W_E} \times 100\%, \quad (7)
\]

\[
\eta_{E+iS} = \frac{PV_{H2}}{RT} \times \frac{\Delta H_{H2}}{W_E + m_S \Delta H_S} \times 100\%, \quad (8)
\]

where \(V_{H2}\) (L) is the volume of produced H₂; \(C_{VS}\) (%) is the volatile solid content of the WH sample; \(V_R\) (L) is the working volume of the sDFMEC reactor; \(t\) (h) is operating time; \(W_E\) (kJ) represents the total electrical energy input into sDMFEC as recorded by the EC-Lab package; \(R\) is the gas consumption rate; \(\Delta H_{H2}\) (kJ/g-TS) is the heat of reaction of H₂ production; \(m_S\) is the volatile solid content of the WH sample; \(\Delta H_S\) (kJ/g-TS) represents the heat of reaction of the heat treatment method.
constant; $P$ (atm) and $T$ (K) represent the pressure and temperature of the biogas, respectively; $\Delta H_{H_2}$ and $\Delta H_S$ represent the heat energies of $H_2$ ($\Delta H_{H_2} = 285.83 \text{ kJ/mol}$ [30]) and WH sample ($\Delta H_S = 15.6 \text{ kJ/g}$), respectively; and $m_c$ (g TS) is the amount of WH added.

3. Results and Discussion

3.1. Effect of Applied Voltage Mode. Firstly, to determine the optimal value of applied voltage for sDFMEC, different applied voltages were investigated in the continuous power input mode (i.e., $C\cdot E_{\text{app}}$), as presented in Figure 2. In theory, an external voltage of around 0.14 V is required for $H_2$ production from acetate-fed MEC [30]. However, no current was observed in WH-fed sDFMEC at an $E_{\text{app}}$ less than 0.2 V, suggesting higher potential losses in sDFMEC associated with the more complex substrate used. Under low $E_{\text{app}}$, $H_2$ was only produced by the activities of fermentative bacteria; thus, a poor $H_2$ yield ($74.5 - 83.3 \text{ mL/g-VS}$) and rate ($3.1 - 3.8 \text{ mL/L/d}$) were achieved. When $E_{\text{app}}$ increased to 0.4 V, the current started to be recorded, and $H_2$ productivity was also significantly increased, around two times higher than that under $E_{\text{app}}$ at 0-0.2 V, indicating that the activity of exoelectrogens in the MEC stage consumed SMPs at the anode and produced $H_2$ at the cathode. However, a large amount of $CH_4$ was formed at $92.2 \pm 4.6 \text{ mL/g-VS}$ (corresponding to $33.1 \pm 4.8 \% \text{ in the produced biogas}$). This indicated that $CH_4$ formation in sDFMEC is caused by hydrogenotrophic ($H_2$ consuming) and electrotrophic (proton consuming) methanogens rather than acetoclastic methanogens (acetate consuming).

The current and $H_2$ productivity also showed a significant increase with increasing $E_{\text{app}}$. As shown in Figure 2(c), the $H_2$ productivity increased almost linearly when the voltage increased from 0.4 to 0.8 V. Specifically, under an $E_{\text{app}}$ of 0.8 V, the $H_2$ yield and rate achieved at $550.2 \pm 16.5 \text{ mL/g-VS}$ and $42.9 \pm 1.3 \text{ mL/L/d}$ which were 7.4 and 13.8 times higher than those without energy input. This $H_2$ yield was almost similar to that obtained from the two-stage DF-MEC process ($565.8 \text{ mL/g-VS}$ [6]); however, the HPR rate nearly doubled ($33.4 \text{ mL/L/h}$ vs. $18.2 \text{ mL/L/h}$ [6]) due to the DF and MEC simultaneously taking place in sDFMEC. The improvement in HPR also contributed to the reduction of $CH_4$ formation due to minimizing the dissolution of $H_2$ into the electrolyte [31, 32]. Accordingly, the $CH_4$ yield of $52.6 \pm 3.5 \text{ mL/g-VS}$ (corresponding to around 9.0% $\text{in the produced biogas}$) was detected under $E_{\text{app}}$ of 0.8 V, reducing approximately 40% when compared to under $E_{\text{app}}$ of 0.4 V.

Table 2 compares the performance of WH-fed sDFMEC to MEC fed with acetate or DF effluent using the same cathode material (i.e., stainless steel mesh SUS304) under an $E_{\text{app}}$ of around 0.8 V. Although it is difficult to make a fair comparison due to differences in experimental setups, especially structural design (e.g., reactor’s volume and electrode’s surface area), it can be seen in Table 2 that WH-fed sDFMEC exhibited a lower peak current density and $H_2$ production rate. This is due to the complex composition of WH
increased energy requirements and reduced metabolic activity of exoelectrogens in WH-fed sDFMEC. Notwithstanding, under an external voltage of 0.8 V, the $H_2$ yield obtained in WH-fed sDFMEC was close to that of MECs fed with simpler substrates, indicating the coproduction of $H_2$ from the DF stage.

When the applied voltage was pushed up to 1.0 V, the current response and $H_2$ production barely increased (Figures 2(a)–2(c)), indicating the rate constraints in the DF and/or MEC stage. Meanwhile, as shown in Figure 2(d), the energy efficiency at $E_{app}$ of 1.0 V continued to decline dramatically because more power was used at higher applied voltages.

**Figure 2:** The performance of WH-fed sDFMEC in C-$E_{app}$ mode at various applied voltages: (a) current response profile (error bars are not shown for visualization), (b) gas production profile (solid and open scatters represent $H_2$ and $CH_4$, respectively), (c) gas yield, and (d) energy recovery. Each data point represents the triplicated experiment.

**Table 2:** Comparison of the performance of MEC and sDFMEC using stainless steel mesh SUS304 as the cathode.

<table>
<thead>
<tr>
<th>Process</th>
<th>Substrate</th>
<th>$E_{app}$ (V)</th>
<th>Peak current density $\alpha$ (mA/cm$^2$)</th>
<th>$H_2$ yield (mL/g)</th>
<th>HPR (mL/L/h)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEC</td>
<td>Acetate (1 g/L)</td>
<td>0.9</td>
<td>$\sim$2.2</td>
<td>1134.9$^b$</td>
<td>87.5</td>
<td>[33]</td>
</tr>
<tr>
<td>MEC</td>
<td>Acetate (1 g/L)</td>
<td>0.8</td>
<td>2.5</td>
<td>512.3</td>
<td>92.1$^b$</td>
<td>[15]</td>
</tr>
<tr>
<td>MEC</td>
<td>DF effluent (1.1 g-SMPs/L)</td>
<td>0.8</td>
<td>1.52</td>
<td>498.7</td>
<td>78.6</td>
<td>[6]</td>
</tr>
<tr>
<td>MEC</td>
<td>DF effluent (1.64 g-SMPs/L)</td>
<td>0.8</td>
<td>1.61</td>
<td>560.8</td>
<td>105.1$^b$</td>
<td>[5]</td>
</tr>
<tr>
<td>sDFMEC</td>
<td>Pretreated WH (5 g-TS/L)</td>
<td>0.8</td>
<td>0.82</td>
<td>550.2</td>
<td>33.4</td>
<td>This study</td>
</tr>
</tbody>
</table>

$^a$Based on the cathode surface area; $^b$calculated from the data provided in the paper.
It can be concluded that the optimal external voltage for WH-fed sDFMEC is 0.8 V under the C-Eapp mode. Therefore, this optimal value of Eapp was further investigated in the I-Eapp mode.

Figure 3 illustrates the performance of WH-fed sDFMEC under I-Eapp mode with three different duty times (1, 60, and 720 minutes, referred to as I-Eapp-1, I-Eapp-60, and I-Eapp-720, respectively). The switch “on” time to switch “off” time ratio was set to 1:1. It can be observed that the temporary maximum current appeared immediately after the input energy source was connected in the switched-on part, suggesting the quick discharge of accumulated electrons, which is consistent with the previous studies [26, 27, 34]. During the “on” period, the current response slightly changes but then suddenly drops to zero once after turning off the input power (Figure 3(a)).

Figures 3(b) and 3(c) present the WH-fed sDFMEC’s performance under the I-Eapp mode with varied duty times compared to the C-Eapp mode. It can be seen that the I-Eapp mode positively affected H₂ production and energy recovery; however, it did not significantly contribute to the suppression of CH₄ formation in sDFMEC. Accordingly, the H₂ yield increased from $550.2 \pm 35.1 \text{ mL/g - VS}$ under C-Eapp to $583.6 \pm 40.3 \text{ mL/g - VS}$ under I-Eapp-1 (Figure 3(b)). The energy recovery was also improved (18.0–38.1% in $\eta_E$) than the continuous voltage application (Figure 3(c)), suggesting the I-Eapp mode is a more practical and efficient option than the C-Eapp mode. As a result of this improvement, the intermittent mode reduced the associated H₂ bubbles on the cathode’s surface, thereby lowering the partial pressure of H₂ and increasing the cathode’s active surface area [27]. Moreover, employing the I-Eapp has been reported to encourage the formation of exoelectrogen biofilm on the anode’s surface, lowering the internal resistance and thereby boosting the process’s efficiency [17, 35]. Notably, the amount of CH₄ was detected at approximately 6.5% (Figure 3(b)) in biogas, reducing the process’s energy efficiency. This indicates that applying the I-Eapp mode cannot
effectively inhibit CH₄ formation; therefore, to improve the performance of WH-fed sDFMEC, other methods to effectively suppress the activity of unwanted microorganisms need to be further investigated and applied. Figure 3 further revealed that the WH-fed sDFMEC’s performance did not change considerably when the duty time was increased in the range of 1–60 minutes. In practice, the I-Eapp-60 will be more useful since shorter duty periods (e.g., I-Eapp-1) may cause more damage to external energy supplies and controllers than longer duty lengths. However, when the duty period was raised to 12 hours (i.e., I-Eapp-720), the performance of the sDFMEC dropped. The reason might be that turning off the external power source for 6 hours could interfere with the metabolic activities of exoelectrogens.

3.2. Effect of Different Methanogens Inhibition Methods. Figure 4 represents the current response and gas profile of sDFMEC under different methanogen inhibition methods, including air exposure (30 minutes, room temperature of 31 ± 1 °C), heat treatment (100°C for 15 minutes), and CHCl₃ (20 ppm). The results showed that the air-exposure method had the lowest inhibitory effect, with a high CH₄ content in biogas of 12.4% by the end of the first cycle and increasing to 23.8% by the end of the fourth cycle (equivalent to around 240 operation hours). This inefficiency can be attributed to the biofilm matrix’s complex structure, preventing the penetration of oxygen [22]. Furthermore, oxygen may cause damage to the other anaerobic exoelectrogens [36], reducing the performance of sDFMECs (Figure 4(b)).

As can also be seen in Figure 4(b), the heat treatment method could inhibit the CH₄ production in sDFMEC, with only 2.2% of CH₄ detected in the produced biogas in the first batch (the 5th in Figure 4(a)); however, it significantly decreases sDFMEC’s performance (YH₂ of only 150.9–238.2 mL/g-VS in the three first batches), indicating a negative impact on the activity of exoelectrogens. Although the...
current and H2 productivity increased almost linearly in the subsequent cycles after heat treatment, CH4 formation also significantly recovered. Specifically, after four cycles (around 240 hours of operation), CH4 occupied over 11% of the produced biogas, suggesting heat treatment is no longer effective and must be repeated. As known, heat treatment does not completely remove spore-forming homoacetogens and methanogens [21, 37], and they, thus, survive and continue to grow when the conditions are favorable, such as a high H2 concentration and a neutral pH in sDFMEC.

CHCl3 addition, on the other hand, exhibited the highest CH4 inhibitory effect and H2 productivity. As is known, CHCl3 has been demonstrated to prevent both methyl-coenzyme M reductase and corrinoid enzymes in methanogens [38]. Thus, the use of CHCl3 can efficiently suppress both methanogens and homoacetogens. However, this study did not achieve complete inhibition of CH4 formation in WH-fed sDFMEC. Around 0.5-1% v/v of CH4 was consistently found even when CHCl3 concentrations were elevated up to 100 ppm (higher concentrations are not recommended to avoid ecosystem toxicity [39], data not shown). The reason may be due to the poor mixing and high H2 concentration at the top part of the sDFMEC reactor, suggesting that the sDFMEC reactor and gas collection system must be improved to get pure H2 in sDFMEC fed with WH. Even so, it can also be observed in Figure 4 that the inhibition effect of CHCl3 was maintained for over 350 operation hours (as shown in the 9th to 15th cycles of Figure 4(a)) in the absence of further CHCl3 injections. Thereby, the high YH2 value of 658.1 ± 14.3 mL/g – VS was obtained in the first

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control condition (C-E app and without CHCl3)</th>
<th>Boosting condition (I-E app, 60 and 20 ppm CHCl3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial TCOD (mg-COD/L)</td>
<td>7621 ± 108</td>
<td>7621 ± 108</td>
</tr>
<tr>
<td>Initial SCOD (mg-COD/L)</td>
<td>2310 ± 45</td>
<td>2310 ± 45</td>
</tr>
<tr>
<td>Final TCOD (mg-COD/L)</td>
<td>5611 ± 60</td>
<td>5463 ± 66</td>
</tr>
<tr>
<td>Final SCOD (mg-COD/L)</td>
<td>348 ± 13</td>
<td>319 ± 8</td>
</tr>
<tr>
<td>TCOD removed (mg-COD/L)</td>
<td>2010 ± 48</td>
<td>2158 ± 42</td>
</tr>
<tr>
<td>SCOD removed (mg-COD/L)</td>
<td>1962 ± 32</td>
<td>1991 ± 37</td>
</tr>
<tr>
<td>H2 produced (mL/g-VS)</td>
<td>431.4 ± 8.8</td>
<td>670.1 ± 15.2</td>
</tr>
<tr>
<td>H2 produced (mg-COD/L)</td>
<td>1214 ± 25</td>
<td>1886 ± 43</td>
</tr>
<tr>
<td>CH4 produced (mL/g-VS)</td>
<td>63.9 ± 5.4</td>
<td>7.0 ± 0.9</td>
</tr>
<tr>
<td>CH4 produced (mg-COD/L)</td>
<td>719 ± 61</td>
<td>79 ± 10</td>
</tr>
<tr>
<td>Total gas produced (mg-COD/L)</td>
<td>1933 ± 86</td>
<td>1965 ± 53</td>
</tr>
<tr>
<td>COD mass balance (%)</td>
<td>96.2 ± 4.6</td>
<td>91.0 ± 2.5</td>
</tr>
</tbody>
</table>

Table 4: Comparison of H2 production from WH in various processes.

<table>
<thead>
<tr>
<th>Process</th>
<th>Pretreatment method</th>
<th>YH2 (mL/g-VS)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>DF</td>
<td>None</td>
<td>30.4</td>
<td>[45]</td>
</tr>
<tr>
<td>DF</td>
<td>Microwave-assisted dilute H2SO4+cellulase</td>
<td>134.9</td>
<td>[46]</td>
</tr>
<tr>
<td>DF</td>
<td>Steam + microwave heating/alkali+cellulase</td>
<td>76.7</td>
<td>[44]</td>
</tr>
<tr>
<td>DF</td>
<td>Microwave-assisted dilute H2SO4+cellulase</td>
<td>112.3</td>
<td>[3]</td>
</tr>
<tr>
<td>DF</td>
<td>Microwave heating/alkali+cellulase</td>
<td>63.9</td>
<td>[47]</td>
</tr>
<tr>
<td>DF</td>
<td>Alkaline+cellulase</td>
<td>51.7</td>
<td>[48]</td>
</tr>
<tr>
<td>DF</td>
<td>Ultrasonic-assisted alkaline</td>
<td>110.4 ± 4.3</td>
<td>[6]</td>
</tr>
<tr>
<td>DF-MEC</td>
<td>None</td>
<td>67.7</td>
<td>[4]</td>
</tr>
<tr>
<td>DF-MEC</td>
<td>Ultrasonic-assisted alkaline</td>
<td>565.8 ± 10.3</td>
<td>[6]</td>
</tr>
<tr>
<td>DF-MEC</td>
<td>Ultrasonic-assisted alkaline</td>
<td>560.8 ± 10.8</td>
<td>[5]</td>
</tr>
<tr>
<td>DF-PF</td>
<td>Steam+microwave heating/alkali+cellulase</td>
<td>596.1</td>
<td>[44]</td>
</tr>
<tr>
<td>DF-PF</td>
<td>Microwave-assisted dilute H2SO4+cellulase</td>
<td>751.5</td>
<td>[3]</td>
</tr>
<tr>
<td>MEC</td>
<td>None</td>
<td>41.4</td>
<td>[4]</td>
</tr>
<tr>
<td>sDFMEC</td>
<td>Ultrasonic-assisted alkaline</td>
<td>670.1 ± 15.2</td>
<td>This study</td>
</tr>
</tbody>
</table>

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seven cycles before decreasing to 602.0 mL/g-VS at the next cycle (16th cycle in Figure 4(a)). This indicated that an occasional dose of CHCl₃ can still effectively prevent CH₄ formation, lowering the process’s chemical costs.

A boosting condition with CHCl₃ addition (20 ppm) and intermittent energy input (I-E_app=60) considerably improved the performance of WH-fed sDFMEC (as shown in the 17th to 25th cycles of Figure 4(a)). Accordingly, compared to the control condition (i.e., C-E_app and without CHCl₃ addition), approximately 1.6 times in H₂ yield (from 431.4 ± 8.8 mL/g – VS to 670.1 ± 15.2 mL/g – VS) and overall energy recovery (from 21.7 ± 0.8% to 34.8 ± 0.5%) were improved (Figure 4(b)). Even so, there was no significant difference in COD removal (TCOD removal of 27% and SCOD removal of 85%) between the boosting and control conditions, as shown in Table 3. This result indicated that the combination of intermittently applied voltage and CHCl₃ addition had a major impact on limiting CH₄ formation, ensuring high efficiency of H₂ production in WH-fed sDFMEC. Table 3 also presents the mass balance for COD of WH-fed sDFMEC, which has a high closure value (91–96%), suggesting the dependability of the experimental data. The attachment of fermentative bacteria to the anode biofilm and/or the detachment of exoelectrogens from the anode can cause these errors in the COD balance.

Table 4 summarizes the H₂ production yield from WH under different processes. It can be seen that DF showed a low efficiency (around 30.4–134.9 mL/g-VS) due to at least two per three parts of hydrogen element being trapped in the formation of SMPs [40, 41]. MEC also exhibited poor performance with WH because the exoelectrogens in MEC prefer simple substrates to polymeric materials or complex wastes [42, 43]. The high content of lignin in fresh WH also causes a low performance of these processes, indicating that the use of effective pretreatment methods is necessary. The combination of DF and MEC or PF can significantly improve the efficiency of H₂ production from WH (560–750 mL/g-VS [3, 5, 6, 44]). In this study, the one-stage process of DF and MEC (i.e., sDFMEC) also showed a high H₂ yield (670.1 ± 15.2 mL/g – VS) which is close to that of the combination of DF and MEC or PF in two-stage cascade processes. However, it should be noted that only a single reactor was applied in sDFMEC, making this process more convenient and cost-effective than other two-stage cascade processes, and its efficiency could also be improved when optimization in structural design is further investigated and applied.

4. Conclusions

This study showed that the single-stage dark fermentation and microbial electrolysis process (i.e., sDFMEC) can be an effective technique for H₂ production from water hyacinth. In WH-fed sDFMEC, the DF and MEC stages occur simultaneously in a single reactor, achieving a comparable H₂ yield to two-stage cascade processes (i.e., DF-MEC or DF-PF). Furthermore, the combination of intermittently applied voltage with the addition of CHCl₃ exhibited a significant improvement in the efficiency of sDFMEC since they could reduce the input electrical energy and effectively prevent the activity of undesirable microorganisms in sDFMEC. However, to maximize the performance and practicability of sDFMECs fed with WH as well as other lignocellulosic feedstocks, further research on optimizing structural design and more environmentally friendly inhibition methods is required.

Data Availability

The experimental data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work was supported by the Can Tho University (CTU), Vietnam; the Ho Chi Minh City University of Natural Resources and Environment (HCMUNRE), Vietnam; and the Gachon University, Republic of Korea.

References


