Performance of a composite bioactive membrane for \( \text{H}_2 \) production and capture from high strength wastewater

Ana L. Prieto, Louis H. Sigtermans, Baris R. Mutlu, Alptekin Aksan, William A. Arnold* and Paige J. Novak*

In this study, a composite bioactive membrane was developed and tested to generate and capture hydrogen (\( \text{H}_2 \)) during the process of wastewater treatment. Hollow fiber membranes were coated with encapsulated acetogenic bacteria to simultaneously produce and capture \( \text{H}_2 \) from waste feedstocks. Acetogens were encapsulated with cast poly(vinylalcohol) or electrospun microfibers. Under anaerobic conditions the poly(vinylalcohol) and electrospun composite membranes produced an average of 44.6 ± 11.3 mL \( \text{H}_2 \) g\(^{-1}\) hexose (0.33 ± 0.08 mol \( \text{H}_2 \) mol\(^{-1}\) hexose) and 21.2 ± 4.8 mL \( \text{H}_2 \) g\(^{-1}\) hexose (0.16 ± 0.04 mol \( \text{H}_2 \) mol\(^{-1}\) hexose), respectively, and captured 73 ± 12% and 57 ± 11%, respectively, of the total \( \text{H}_2 \) produced in bioreactors fed synthetic high strength wastewater. The \( \text{H}_2 \) capture efficiency of the electrospun composite membrane was improved by coating the modules with a thin film of polymeric silica gel, improving the \( \text{H}_2 \) production to 28.3 ± 2.3 mL \( \text{H}_2 \) per hexose (0.21 ± 0.02 mol \( \text{H}_2 \) mol\(^{-1}\) hexose) and the \( \text{H}_2 \) capture efficiency to 73 ± 15%. Final composite membranes were built by immobilizing bacteria directly onto the membrane surface, again improving \( \text{H}_2 \) yields from high strength synthetic wastewater to a maximum of 48.4 ± 9.4 mL \( \text{H}_2 \) g\(^{-1}\) hexose (0.36 ± 0.07 mol \( \text{H}_2 \) mol\(^{-1}\) hexose) with a maximum \( \text{H}_2 \) capture efficiency of 86 ± 9%. The optimized composite membranes were also capable of generating and capturing \( \text{H}_2 \) from real wastewaters, with yields and capture efficiencies of 19.2 ± 3.0 mL \( \text{H}_2 \) g\(^{-1}\) hexose (0.14 ± 0.02 mol \( \text{H}_2 \) mol\(^{-1}\) hexose) and 99.1 ± 0.2%, and 46.0 ± 15.5 mL \( \text{H}_2 \) g\(^{-1}\) hexose (0.34 ± 0.12 mol \( \text{H}_2 \) mol\(^{-1}\) hexose) and 79 ± 19% when tested with a feed of sugar beet wastewater and dairy production wastewater, respectively. After further optimization, the composite membrane system could allow the extraction of high-quality energy from wastewater.

Water impact

This investigation will benefit society by decreasing wastewater treatment costs and energy use in centralized and decentralized applications. Additional benefits include the production of clean energy from marginal streams and reduction of waste strength upstream of conventional treatment trains. Reduction of chemical input, treatment energy requirements and overall carbon and energy footprint of the wastewater treatment process, are potential outcomes from the application of this technology.

Introduction

Despite the inherent chemical energy potential of wastewater, current wastewater treatment practices expend a considerable amount of energy to remove dissolved energy-dense compounds. Indeed, the water and wastewater treatment sectors account for 3–4% of the energy use in the United States,\(^1\) which is similar to that in other developed countries.\(^2\) A typical municipal wastewater treatment plant allocs more than 50% of its total energy use to aeration,\(^2\) converting the reduced chemical energy within wastewater into \( \text{CO}_2 \) and biomass. Opportunities to convert waste to energy in wastewater treatment are abundant, resulting in energy neutral or even energy generating treatment plants.\(^3\)

Anaerobic digestion is used primarily to harvest energy in the form of methane biogas from high strength wastewaters.
By inhibiting naturally occurring methanogens that consume hydrogen (H₂) and acetate during anaerobic digestion, however, it is possible to redirect the degradation of the dissolved organic compounds in wastewater to produce H₂. H₂, when produced biologically, is regarded as a renewable and attractive clean energy source as a result of its high energy density and clean-burning properties. Nevertheless, technical considerations such as a need for stringent pH control and microbial competition limit the deployment of waste-to-H₂ reactors. Previous systems designed for H₂ production from wastewater have typically required pretreatment of the influent wastewater to deactivate H₂-consuming methanogens. Pretreatment techniques include heating, acidifying, or autoclaving the wastewater, which reduces the net energy gained from the process and is not likely to be feasible at a realistic scale. The isolation and protection of H₂-producing acetogens through encapsulation could provide a solution to this competition problem without the need for waste pretreatment. Many chemistries for microbial encapsulation or immobilization have been explored, including sol–gel polymers, silica gel nanoparticles, latex-coatings, and electrospun fibers, all while demonstrating cell viability. With such methods, the encapsulation of acetogens could enable the spatial control of these populations and could also separate and isolate them from methanogens. Indeed, methods of immobilizing acetogens for H₂ production have been extensively studied. What has been missing from such studies, however, is an efficient mechanism for removing the H₂ once it is produced.

Because H₂ production is less favorable when the H₂ partial pressure is high, removing excess H₂ from the liquid phase as it is produced is critical. Some approaches to removing H₂ include absorption of H₂ in metals (e.g., Pd and LaNi₅) or stripping H₂ by boiling, recirculating a gas stream through the reactor (e.g., N₂, CO₂, steam), or allowing evaporation at a surface. These approaches, again, are all likely to result in significant operational costs at the scale required. By providing a high surface area for gas transfer, hollow fiber membranes offer a modular, energy efficient method to capture and remove H₂ from wastewater. Some studies have used hollow fiber membranes for H₂ removal in aceticogenic reactors, reducing the partial pressure of H₂, and consequently improving the H₂ production rate (volume of H₂ per day) and H₂ yields (volume of H₂ per g hexose or mol H₂ mol⁻¹ hexose). To maximize H₂ capture, however, it is also necessary to prevent the growth of methanogenic bacteria, which would consume H₂.

In this study, simultaneous H₂ production and capture from wastewater, building upon the concepts of membrane gas transfer and microbial encapsulation, is reported. Encapsulated aceticogenic bacteria and hollow fiber membranes are used to create a composite membrane module wherein H₂ producing bacteria are immobilized in close proximity to hollow fiber membranes that enable gas collection and removal as it is produced (Fig. 1). To our knowledge, this is the first technology that allows simultaneous and efficient production and capture of H₂ from wastewater. To achieve both proof-of-concept and optimization of the technology, the composite membrane module was built using different encapsulation methods and material chemistries. The membranes were then tested in synthetic wastewater to demonstrate their potential for H₂ production and capture. Further, the membranes were tested with real high strength wastewaters from dairy and sugar beet production to demonstrate the applicability of the technology with actual waste streams.

Materials and methods

2.1. Feedstock and microbial seed

Synthetic wastewater was prepared as described in Klatt and LaPara (2003) and modified to increase chemical oxygen demand (COD) content. Dairy production wastewater was obtained from the permeate line of a microfiltration unit in a local dairy production plant and contained 3.72% lactate. To avoid overloading the reactors, the dairy wastewater was diluted 10-fold, to an average soluble COD of 7.8 ± 0.3 g L⁻¹, before feeding the reactors. The sugar beet wastewater was collected from a retention pond in a local sugar beet production facility. The sugar beet wastewater had a COD of 37 g L⁻¹ and was also diluted 10-fold before feeding.

An aceticogenic seed culture was obtained by heat-treating a sample of municipal anaerobic sludge at 95 °C for 40 minutes. Serum bottles containing synthetic wastewater were inoculated with heat-treated sludge and incubated at 36 °C for 24 hours. Incubated cultures were washed with DI water twice and concentrated through centrifugation. Additional seed cultures enriched on the dairy or sugar beet wastewater were also obtained by inoculating serum bottles containing the target waste with heat-treated sludge, and further allowing them to acclimate for a period of 30 days.
2.2. Bioreactor and membrane construction

The experimental set-up consisted of a 2.75 L completely mixed anaerobic reactor containing a submerged composite membrane module (Fig. 2). The reactor was continuously fed with a peristaltic pump (Masterflex 7520-25, Cole Palmer, Vernon Hills, IL) and the hydraulic residence time was maintained at 18 h. The average influent flow rate \( Q \) was \( 2.5 \pm 0.2 \text{ mL min}^{-1} \). Influent and effluent pH were monitored daily and the reactor’s pH was adjusted to 4.5–5.5 using NaHCO₃. Influent and effluent COD were monitored 3 times per week. The membrane module was plumbed into a gas line fed by compressed ultra high purity N₂ that flowed into and out of the module continuously to sweep out the biologically produced gas \( (e.g., \text{H}_2) \). The gas flow rate \( (Q_{\text{g-off}}) \), measured daily, was controlled manually with a gas flow meter and a needle valve and maintained at 10 mL min⁻¹. The gas loss to the headspace \( (Q_{\text{g-off}}) \) was measured daily using volume displacement. The composition \( (\text{H}_2 \text{ and CH}_4 \text{ content}) \) of \( Q_{\text{g-off}} \) and the dissolved gas exiting the reactor was measured daily by taking a 5 mL sample of effluent with a gas-tight syringe.

\[ Q = Q_{\text{g-off}} + Q_{\text{g-in}} + Q_{\text{g-out}} \]

where \( Q \) is the influent liquid flow, \( Q_{\text{g-off}} \) is the flow rate of gas exiting the reactor, \( Q_{\text{g-in}} \) and \( Q_{\text{g-out}} \) is the sweep gas flow through the membranes. Note that the solid line refers to the liquid flow and the dashed is the gas flow through and out the membrane module.

\[ \text{flow, } \text{g-off}, \text{ g-in, g-out} \]

\[ \text{out, } \text{N}_2 + \text{H}_2 \]

\[ \text{Food pump, } \text{Membrane module, } \text{Captured gas sample} \]

**Fig. 2** Schematic of the experimental set-up. \( Q \) is the influent liquid flow, \( Q_{\text{g-off}} \) the flow rate of gas exiting the reactor, \( Q_{\text{g-in}} \) and \( Q_{\text{g-out}} \) is the sweep gas flow through the membranes. Note that the solid line refers to the liquid flow and the dashed is the gas flow through and out the membrane module.

\[ \text{aqueous solution of PVA; Elivanol 71-30 DuPont; Wilmington, DE). Concentrated microbial seed (approximately 4.7 mg in 1 mL) was mixed into 30 mL of the PVA solution, after which it was cast onto the support/gas transfer layer. The cast PVA was allowed to dry for 24 hours after which it was cross-linked in a solution of 1% (w/v) aqueous boric acid for 4 minutes. The thickness of the dry PVA coat was ~1 mm. A negative control (abiotic) membrane module was also constructed that was identical to module M1 except that the PVA layer did not contain cells.

2.2.1.1 Encapsulation using cast PVA. The bioactive layer for membrane modules 2 and 3 (M2 and M3) consisted of the aceticogenic seed culture encapsulated in electrospun microfibers as described in Klein et al. (2009).¹³

Briefly, a core polymeric solution containing the seed culture and a shell polymer solution were co-spun using a spinneret with two coaxial capillaries. The core solution consisted of 5 wt% polyethylene oxide 600 K in water. One mL of concentrated seed (approximately 0.3 mg dry weight equivalent) was combined with 10 ml of core solution. The shell solution was 9 wt% polycaproalactone (PCL) 80 K and 1 wt% polyethylene glycol (PEG) 6 K dissolved in a mixture of chloroform and dimethylformamide, 9:1 (w/w). The microfibers were electrospun over the hollow fiber membrane mat for approximately 2 hours on each side, forming a uniform film with a thickness of approximately 0.2 mm.

In module M3, a third layer of silica gel was added on top of the electrospun layer. The silica gel was composed of tetraethyl orthosilicate (TEOS)-cross-linked silica nanoparticles (TM40) at a 3:1 ratio (v/v), as described in Mutlu et al. (2013).²⁶ The silica gel solution was sprayed on the electrospun layers to a thickness of approximately 0.26 to 0.39 mm before gelling occurred. A negative control (abiotic) membrane module was also constructed that was identical to module M2 except that the electrospun layer did not contain cells.

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2.2.1.3 Encapsulation using poly(dopamine) (PDA) and a polymeric sealing coat. An additional set of membrane modules (M4a, M4b, and M5) were created by immobilizing the aceticogenic seed culture directly onto the membrane surface using PDA (H8502-25G, Sigma Aldrich, St. Louis, MO). The acetogen layer was followed by an additional polymeric layer to act as a seal to protect the organisms and prevent them from releasing back into the reactor bulk. For these three modules, a 2 g L⁻¹ PDA solution was prepared by dissolving dopamine hydrochloride in 10 mM Tris solution (pH 8.5). The bare hollow fiber membrane mats were dipped with a thin film of PDA (<50 nm) to provide an adhesive surface for the cells. Defined volumes of a concentrated seed culture were then spray onto each side of the PDA-coated surface. After air-drying, M4a and M4b were dipped-coated with silica gel. The silica gel was identical to that described in section 2.2.1.2. Module M4a contained approximately 0.2 mg of cell mass, while module M4b contained 0.4 mg of cell mass. For module M5, PVA, containing no cells but otherwise identical to that described above (section
2.2.1.1), was cast over the cells to seal the biological layer. Approximately 0.4 mg of cell mass was immobilized on module M5.

2.3. Analytical methods

Gas flow rates were monitored volumetrically using an inverted graduated cylinder and a timer. COD values were measured in diluted samples using Hach HR COD digestion vials (Hach Company, Loveland, CO). The lower detection limit was 20 mg COD L\(^{-1}\). Measurements of total solids (TS) were performed according to Standard Method 2540.\textsuperscript{27} Biogas composition was measured using GC-TCD (model 6890; Agilent Technologies, Santa Clara, CA) equipped with a packed column, Supelco molecular sieve 13 × 45/60, 10 ft × 1/8 in × 2.1 mm (Sigma-Aldrich, St. Louis, MO). N\(_2\) was used as carrier gas at 20 mL min\(^{-1}\). A gas sample was taken with a locking gas-tight syringe and 200 μL was injected for measurement.

2.4. Data analysis

The total H\(_2\) production in the reactor is expressed as:

\[
Q_{\text{H}_2\text{-tot}} = \phi_{\text{H}_2\text{-out}} Q_{\text{g-out}} + \phi_{\text{H}_2\text{-diss}} Q_{\text{H}_2\text{-diss}} + \phi_{\text{H}_2\text{-off}} Q_{\text{H}_2\text{-off}}
\]

where \(Q_{\text{H}_2\text{-tot}}\) is the total H\(_2\) produced in the reactor, \(\phi_{\text{H}_2\text{-out}}\) is the measured volume fraction of H\(_2\) exiting the reactor, \(Q_{\text{g-off}}\) is the flow rate of gas exiting the reactor, \(\phi_{\text{H}_2\text{-diss}}\) is the measured volume fraction of H\(_2\) dissolved in the effluent, \(Q\) is the effluent flow rate, \(k_{\text{H}_2}\) is the specific dimensionless Henry’s law constant, \(\phi_{\text{H}_2\text{-out}}\) is the measured volume fraction of H\(_2\) captured by the membrane, and \(Q_{\text{g-out}}\) is the flow rate of sweep gas through the membranes. All flow rates are reported in units of mL day\(^{-1}\).

The performance of the membrane module was evaluated based on the H\(_2\) production/capture rate (\(\phi_{\text{H}_2\text{-out}} Q_{\text{g-out}}\)). To calculate the H\(_2\) yield (\(Y_H\)), the H\(_2\) production/capture rate is normalized to the sugar or hexose content in the feed wastewater (eqn (2)).

\[
Y_H = \frac{\phi_{\text{H}_2\text{-out}} Q_{\text{g-out}}}{C_{\text{hex}} Q}
\]

where \(C_{\text{hex}}\) is the hexose/sugar concentration of the feedstock, \(Q\) is the feed flow rate, and \(Y_H\) is the H\(_2\) capture yield in mL g\(^{-1}\) hexose (or mol H\(_2\) mol\(^{-1}\) hexose). Additionally, the H\(_2\) capture efficiency of each module (\(\eta\)) is calculated as described in eqn (3).

\[
\eta = \frac{\phi_{\text{H}_2\text{-out}} Q_{\text{g-out}}}{Q_{\text{H}_2\text{-at}}}
\]

Values of H\(_2\) yield from the literature were compared to the values determined here. Eqn (4) was derived from the temperature coefficient (\(Q_{10}\)) expression\textsuperscript{28} and was used to account for temperature differences among studies in the literature.

\[
Y_{22} = \frac{Y_T}{T_{22}} \left(\frac{Q_{10}}{10}\right)
\]

where \(Y_T\) is the observed yield, \(T\) is the operating temperature of the study in question, and \(Y_{22}\) is the estimated yield at 22 °C. For most biological processes, \(Q_{10}\) values between 2 to 3 are used.\textsuperscript{28} A value of 2.5 was used in this study.

The contribution of H\(_2\) from the mixed liquor to the membrane was calculated using eqn (5). At steady state, the flux (\(j\), mol time\(^{-1}\)) of H\(_2\) from the mixed liquor to the membrane lumen can be as described as follows:

\[
j = \frac{DH}{l} \left(\frac{C_{\text{up}} - C_{\text{down}}}{k_{\text{H}_2}}\right)
\]

where \(D\) is the diffusion coefficient of the membrane polymer (m\(^2\) s\(^{-1}\)), \(H\) (dimensionless) is the partition coefficient between the membrane and adjacent solution (i.e., mixed liquor), \(l\) is the membrane thickness, \(C_{\text{up}}\) is the H\(_2\) concentration upstream of the membrane (i.e., mixed liquor), and \(C_{\text{down}}\) is the downstream H\(_2\) concentration. For reasons described below, \(C_{\text{up}}\) was assumed to be the saturation concentration of H\(_2\) gas in water (0.8 mM). In this study, ultra pure N\(_2\) gas was used as sweep gas, thus the concentration of H\(_2\) downstream of the membrane (i.e., lumen) is zero (\(C_{\text{down}} = 0\)). Therefore, eqn (5) can be simplified as:

\[
\frac{DH}{l} \frac{C_{\text{g-off}}}{k_{\text{H}_2}}
\]

By assuming that the uncoated polyethylene hollow fibers were stripping H\(_2\) from the bulk liquid saturated with H\(_2\) gas, a “worst-case scenario” ratio of H\(_2\) produced by the encapsulated bacteria compared to that stripped from bulk solution could be calculated. This value provides the maximum stripping that can occur in a given situation and thereby provides the most conservative value for the biologically produced H\(_2\) within the composite membrane. For the polyethylene hollow fibers, \(D\) and \(H\) values were 4.74 × 10\(^{-11}\) m\(^2\) s\(^{-1}\) and 1.73 × 10\(^{-1}\), respectively.\textsuperscript{28} The membrane thickness and area were 50 μm and 0.015 m\(^2\), respectively.

Results

3.1. Proof-of-concept of the composite bioactive membrane for H\(_2\) production and capture

Results from experiments with modules M1 and M2, used to compare two different immobilization methods and polymer chemistries (i.e., cast PVA and electrospun fibers), are shown in Fig. 3 and 4. M1 was operated for more than 30 days, reaching stable operation and steady H\(_2\) production after a 4 day acclimation period. Operational variables such as
influent pH and $Q_{g-in}$ were varied between 6.5–8.5 and 2–8 mL min$^{-1}$, respectively, to identify their effect on membrane performance (Fig. 3). With influent pH held constant, a higher $Q_{g-in}$ of 8 mL min$^{-1}$, compared to 2 mL min$^{-1}$, resulted in a higher H$_2$ yield ($p < 0.05$). At a constant gas flow rate, a feed pH of 8.5, compared to 6.5, also resulted in a higher H$_2$ yield ($p < 0.05$). During stable operation, module M1 produced $290.1 \pm 59.5$ mL H$_2$ day$^{-1}$ with an average yield of $44.6 \pm 11.3$ mL H$_2$ g$^{-1}$ hexose. H$_2$ was also produced in the bioreactor and lost with the reactor’s liquid effluent, with only $73 \pm 12\%$ of the produced H$_2$ captured by the composite membrane module. Module M1 contained 4.7 mg of encapsulated bacteria, producing approximately 61.6 mL H$_2$ d$^{-1}$ mg$^{-1}$ biomass.

Module M2, with the electrospun fibers, was operated for more than 30 days with $Q_{g-in}$ at 8–10 mL min$^{-1}$. After an acclimation period of 1 week, H$_2$ generation/capture was observed. During stable operation (DAYS 10–30) the average yield was $21.2 \pm 4.8$ mL H$_2$ g$^{-1}$ hexose ($0.16 \pm 0.04$ mol H$_2$ mol$^{-1}$ hexose) with an average of $132.6 \pm 29.3$ mL H$_2$ d$^{-1}$ captured. This was less than that observed in module M1; nevertheless, module M2 contained only 0.3 mg of cells, 6% of the cell mass contained in module M1, suggesting that cell density is an important variable in this system. Interestingly, module M2 produced 442.1 mL H$_2$ d$^{-1}$ mg$^{-1}$ biomass. This was seven times more H$_2$ per mg biomass than observed in module M1, indicating that, despite the lower overall quantity of H$_2$ generated over time, the electrospun system allowed for more H$_2$ production per mg biomass, perhaps as a result of better viability post-encapsulation or better diffusion of substrate to the cells. Finally, the capture efficiency of module M2 was only $57 \pm 11\%$, resulting in a H$_2$ concentration of $1.0 \pm 0.2\%$ (v/v) in the module off-gas and a large fraction of the produced H$_2$ lost to the liquid reactor effluent. Compared to module M1, the electrospun fibers in module M2 detached from the hollow fiber mat, resulting in poor contact between the encapsulated acetogens and the hollow fibers.

Experiments with modules M1 and M2 showed that H$_2$ was lost from the system via two mechanisms: diffusion of H$_2$ out of the module and into the reactor liquid and production of H$_2$ outside of the bioactive composite membrane as a result of the presence of H$_2$-producing bacteria in the bulk.

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Fig. 3  H$_2$ yield and reactor pH during experiment with module M1. Changes in N$_2$ influent flow ($Q_{g-in}$) and influent feed pH are indicated at the top.

Fig. 4  Summary of H$_2$ yield and H$_2$ capture efficiencies of the different membrane modules tested in this study. The bottom boxes indicate the different construction methods and encapsulating medium used. M1 = poly(vinyl) alcohol (PVA) + hollow fibers (HF), M2 = e-spun + HF, M3 = e-spun + HF + silica coat, M4a = HF + 1×(polydopamine (PDA) + cell coat) + silica gel seal, M4b = HF + 2×(PDA + cell coat) + silica gel seal, and M5 = HF + 2×(PDA + cell coat) + PVA seal.
solution. Negative control experiments with PVA and electrospun layers did not show H₂ production or capture (see Fig. S27), clearly demonstrating the advantage of adding specific H₂-producing organisms to the system. Nevertheless, these organisms were able to leak from the bioactive layer and seed the reactor, where the H₂ generated would not necessarily be captured in a system treating actual waste and containing methanogens. Based on the diffusion calculation described above, modules M1 and M2 were stripping H₂ from the reactor mixed liquor to some extent. Indeed, using eqn (6), about 12% and 26%, of the H₂ flux coming into the lumen of modules M1 and M2 respectively, consisted of H₂ stripped from the reactor bulk liquid via diffusion into the hollow fibers (Table 1). As stated above, this assumes the bulk liquid was saturated with H₂, an assumption supported by observations of bubble formation in the reactor and GC measurements indicating that these bubbles were comprised primarily of H₂. While not necessarily a problem with the laboratory-scale system, if scaled up and operated in this manner with unsterilized industrial waste, the H₂ produced would be quickly consumed in the bulk reactor liquid reducing overall H₂ production and capture.

3.2. Optimization of the composite membrane module

To address the problems of contact, leakage of bacteria from the membrane, and production of H₂ in/loss of H₂ to the bulk liquid, the composite membranes were modified (module M3) by adding a silica gel sealant layer on top of the electrospun-encapsulated cells. Silica gel was used for this purpose because this material can be easily modified, offering flexibility with respect to porosity, permeability, and surface functionality, thereby maximizing the activity of encapsulated bacteria and enhancing transport of substrates into the gel.26,10 During 15 days of operation with synthetic high strength waste, the membrane modules produced 173.8 ± 10.2 mL H₂ day⁻¹ with a yield of 28.3 ± 2.3 mL H₂ per hexose (0.21 ± 0.02 mol H₂ mol⁻¹ hexose). The average H₂ concentration in the out-gas was still low, 1.2 ± 0.1% H₂ (v/v), but the capture efficiency increased to 73 ± 15% (Fig. 4), demonstrating that the sealant layer did improve performance. Neverthe-

<table>
<thead>
<tr>
<th>Membrane module</th>
<th>Measured flow of total captured H₂ (mL per day)</th>
<th>% H₂ flow from bioactive layer</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1 PVA + HF</td>
<td>290.12</td>
<td>88.1%</td>
</tr>
<tr>
<td>M2 e-spin + HF</td>
<td>132.62</td>
<td>74.1%</td>
</tr>
<tr>
<td>M3 e-spin + HF + silica coat</td>
<td>173.79</td>
<td>80.2%</td>
</tr>
<tr>
<td>M4a HF + (PDA + cell coat) + silica gel seal</td>
<td>198.78</td>
<td>82.7%</td>
</tr>
<tr>
<td>M4b HF + 2x(PDA + cell coat) + silica gel seal</td>
<td>251.57</td>
<td>86.3%</td>
</tr>
<tr>
<td>M5 HF + 2x(PDA + cell coat) + PVA seal</td>
<td>272.14</td>
<td>87.4%</td>
</tr>
<tr>
<td>M5 – HRT: 18 hours HF + 10x(PDA + cell coat) + PVA seal</td>
<td>275.81</td>
<td>87.5%</td>
</tr>
<tr>
<td>M5 – HRT: 48 hours HF + 10x(PDA + cell coat) + PVA seal</td>
<td>143.45</td>
<td>76.0%</td>
</tr>
<tr>
<td>M5 – sugar beet waste HF + 10x(PDA + cell coat) + PVA seal</td>
<td>120.91</td>
<td>100%</td>
</tr>
<tr>
<td>M5 – dairy waste HF + 10x(PDA + cell coat) + PVA seal</td>
<td>272.46</td>
<td>87.4%</td>
</tr>
</tbody>
</table>

In spite of the silica gel’s robustness in terms of material stability, when applied as a thin film, poor mechanical strength was observed.21 In wastewater treatment applications this would likely result in the loss of the acetogenic biomass over time. To overcome this problem, module M5 was constructed identically to module M4b, except that PVA was used as a sealant layer rather than silica gel. With 0.4 mg of encapsulated biomass, M5 produced 272.1 ± 37.4 mL H₂ per day, with a yield of 48.4 ± 9.4 mL H₂ g⁻¹ of hexose (0.36 ± 0.07 mol H₂ mol⁻¹ hexose), and a capture efficiency of 71 ± 11%. The contribution of H₂ from the bioactive layer in this case was 87% (Table 1).

M5 demonstrated improved results in terms of H₂ production and capture. Consequently, M5 was tested in dairy production wastewater and sugar beet wastewater to further demonstrate the applicability of the technology to actual waste streams. M5 modules used for these experiments contained biomass acclimated to dairy production wastewater and sugar beet wastewater at 2.0 mg biomass and 4.5 mg biomass, respectively. H₂ production of 272.5 ± 92.0 mL H₂ per...
day, a yield of $46.0 \pm 15.5 \text{ mL H}_2 \text{ g}^{-1} \text{ hexose (0.34 \pm 0.12 mol H}_2 \text{ mol}^{-1} \text{ hexose)}$, and a capture efficiency of $79 \pm 19\%$ were achieved using M5 in dairy wastewater. M5 in sugar beet wastewater produced $120.9 \pm 19.5 \text{ mL H}_2 \text{ per day, 19.1 \pm 3.0 mL H}_2 \text{ g}^{-1} \text{ hexose (0.14 \pm 0.02 mol H}_2 \text{ mol}^{-1} \text{ hexose)}$, and captured $99.0 \pm 0.2\%$ of the total H$_2$ produced. Both modules were operated for more than 15 days, and in the sugar beet and dairy wastewater 100\% and 87\% of the H$_2$, respectively, was produced by the encapsulated bacteria based on the diffusion calculations. In the case of the sugar beet wastewater, the water in the reactor was not saturated with H$_2$ (0.01 mM), leading to a negligible contribution from stripping.

**Discussion**

In addition to providing proof-of-concept data, this study reports a systematic approach to improving the physical characteristics and performance of an experimental technology, a composite bioactive membrane module. Each prototype membrane module proved effective in producing and capturing H$_2$ from high strength wastewater. Module M5, however, demonstrated clear advantages in terms of yield, H$_2$ capture efficiency, and the mechanical robustness of the module, resulting in a design potentially suitable for scale-up. To put this technology in context, the results of M5 were compared to other studies that used suspended cultures to produce H$_2$ from different sources of pretreated waste (Table 2).

Estimated yields at 22 °C in similar studies from the literature, utilizing similar feedstocks, seed cultures, and reactor types, ranged from 45–92 mL H$_2$ g$^{-1}$ hexose (Table 2). Although the H$_2$ yields in this study are within this range (48.43 ± 9.41 mL H$_2$ g$^{-1}$ hexose), the module-based technology proposed herein offers some critical advantages. First, the yield values reported in this study refer to the captured H$_2$ that is readily available for on-site applications (e.g., cogeneration), and not to the total H$_2$ produced in the reactors. While successfully demonstrating H$_2$ production, previous studies have been hampered by technological limitations that result from the inability to easily capture and remove H$_2$ from the system.$^{34,37}$ Additionally, in these previous studies the need to prevent interspecies H$_2$ transfer in non-sterile conditions requires operating with pretreated feedstock or at low pH or short retention times, lowering the net process energy balance or resulting in lower H$_2$ yields.$^{38}$ Lastly, many reactor designs for H$_2$ production are difficult to scale-up from the laboratory to commercial and industrial scales. The proposed technology attempts to overcome these limitations by utilizing a modular system in which the energy required to supply sweep gas to the hollow fiber membrane system is significantly less than sparging$^{39}$ and by utilizing encapsulated H$_2$-producing bacteria to reduce interspecies H$_2$ transfer and eliminate the need for feedstock pretreatment.

Laboratory studies have demonstrated that a range of industrial waste streams are feedstocks for fermentative H$_2$ production, including noodle manufacturing waste,$^{35}$ rice winery wastewater,$^{40}$ filtered leachate of waste biosolids,$^{41}$ sugar beet wastewater,$^{36}$ palm oil mill effluent,$^{42}$ and pig waste slurry.$^{43}$ While using suspended growth, previous technologies were designed to build up enough biomass to provide H$_2$ generation at low HRT. The proposed technology decouples the HRT from the SRT, providing flexibility. At an HRT of 18 hours, the optimized module M5 was able to generate 46.02 mL H$_2$ g$^{-1}$ hexose ($0.34 \pm 0.12 \text{ mol H}_2 \text{ mol}^{-1} \text{ hexose)$ and 19.15 mL H$_2$ g$^{-1}$ hexose ($0.14 \pm 0.02 \text{ mol H}_2 \text{ mol}^{-1} \text{ hexose}$) from dairy production and sugar beet wastewater respectively. Even though these values are less than 50\% of those reported in other studies for similar substrates,$^{36,44}$ they compare favorably to technologies that report the total H$_2$ production of the bioreactor at higher concentrations of biomass.$^{45}$ Indeed, based on the volume of sugar beet wastewater in Minnesota alone, this system, unoptimized, would yield approximately 2630 MW h year$^{-1}$ additional electricity.

This technology is at a very early stage of development and at this time neither material cost nor module manufacture are sufficiently optimized for scale-up. Operational conditions of the reactors also play a key role in the membrane performance. For instance, CH$_4$, CO$_2$ and H$_2$S are likely to be present if the buffering capacity and characteristics of the substrate provides a suitable environment of methanogenic growth. If the organic acids present in the bulk liquid are further degraded to CH$_4$, the overall production of combustible gas will increase, resulting in a mixed CH$_4$-H$_2$ gas stream. Nevertheless, additional cleaning (e.g., wet scrubbing, packed columns) could be necessary to remove H$_2$S or other

<table>
<thead>
<tr>
<th>Carbohydrate substrate</th>
<th>Seed type</th>
<th>Reactor type</th>
<th>Temp (°C)</th>
<th>Yield (mL H$_2$ g$^{-1}$ hexose)</th>
<th>Estimated yield at 22 °C</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>SS</td>
<td>CSTR</td>
<td>36</td>
<td>260</td>
<td>72</td>
<td>32</td>
</tr>
<tr>
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<td>ADS</td>
<td>CSTR</td>
<td>35</td>
<td>148</td>
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<td>33</td>
</tr>
<tr>
<td>Wheat starch</td>
<td>ADS</td>
<td>CSTR-gs</td>
<td>33</td>
<td>254</td>
<td>77</td>
<td>34</td>
</tr>
<tr>
<td>Noodle mfg waste</td>
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<td>CSTR</td>
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<td>200</td>
<td>61</td>
<td>35</td>
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<tr>
<td>Sugar beet wastewater</td>
<td>ADS</td>
<td>CSTR-gs</td>
<td>32</td>
<td>231</td>
<td>92</td>
<td>36</td>
</tr>
<tr>
<td>High strength sewage surrogate</td>
<td>ADS</td>
<td>CSTR-gs</td>
<td>22</td>
<td>48.4</td>
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</tr>
<tr>
<td>Dairy production wastewater</td>
<td>Acclimated ADS</td>
<td>CSTR</td>
<td>22</td>
<td>46.0</td>
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</tr>
<tr>
<td>Sugar beet wastewater</td>
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<td>22</td>
<td>19.1</td>
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<td>This study – M5</td>
</tr>
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</table>
impurities. In addition, the highest H₂ concentration achieved in the membrane off-gas was 2.3% for M5. This would need to be improved, either via the use of vacuum gas collection, metal–organic frameworks for concentrating and storing H₂,⁴⁶,⁴⁷ or improved H₂ production in the bioactive layer. Nevertheless, the results presented with the optimized module M5 are promising and provide another technological opportunity to explore energy-neutral or energy-generating wastewater treatment.

Conclusions

Composite bioactive membrane modules were able to produce and capture H₂ from high-strength synthetic and real wastewaters. This novel approach can potentially overcome many of the problems previously encountered in reactors employing fermentative H₂ production. Indeed, by continuously removing H₂ from the liquid phase, the H₂ partial pressure was maintained below inhibitory values for the acetogenic community in this study. Furthermore, the hollow fiber membranes allowed the off gas to be easily collected, which would facilitate on-site energy generation (e.g., combined heat and power), use in fuel cells, or concentration for industrial use and storage. Although electrospun microfibers appeared to provide more surface area for the diffusion of nutrients and substrate to the encapsulated cells, the density of encapsulated cells was restricted, which in turn restricted the total quantity of H₂ generated.

A multi-layer configuration (i.e., hollow fiber membranes/imobilized cells/sealant layer), together with alternative encapsulation methods (i.e., PDA-immobilized cells) showed promising results in terms of the yield and the H₂ capture efficiency. Future research will focus on increasing the cell density in the bioactive layer, changing the H₂ collection to improve the H₂ concentration in the off-gas (i.e., via vacuum), and exploring alternative materials and manufacture protocols to improve scale-up.

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