

CrossMark
click for updatesCite this: *J. Mater. Chem. A*, 2015, 3,
20698

Artificial leaf device for hydrogen generation from immobilised *C. reinhardtii* microalgae†

Anupam A. K. Das,^a Mohammad M. N. Esfahani,^b Orlin D. Velev,^c Nicole Pamme^a
and Vesselin N. Paunov^{*a}

We developed a fully biomimetic leaf-like device for hydrogen production which allows incorporated fabric-immobilised microalgae culture to be simultaneously hydrated with media and harvested from the produced hydrogen in a continuous flow regime without the need to replace the algal culture. Our leaf device produces hydrogen by direct photolysis of water resulting from redirecting the photosynthetic pathways in immobilised microalgae due to the lack of oxygen. In contrast to the many other reports in the literature on batch photobioreactors producing hydrogen from suspension culture of microalgae, we present the first report where this is done in a continuous manner from a fabric-immobilised microalgae culture. The reported artificial leaf device maximises the sunlight energy utilisation per gram of algae and can be upscaled cheaply and easily to cover large areas. We compared the production of hydrogen from both immobilised and suspended cultures of *C. reinhardtii* microalgae under sulphur, phosphorus and oxygen deprived conditions. The viability and potential of this approach is clearly demonstrated. Even though this is a first prototype, the hydrogen yield of our artificial leaf device is twenty times higher per gram of algae than in previously the reported batch reactors. Such leaf-like devices could potentially be made from flexible plastic sheets and installed on roofs and other sun-exposed surfaces that are inaccessible by photovoltaic cells. The ability to continuously produce inexpensive hydrogen by positioning inexpensive sheets onto any surface could have an enormous importance in the field of biofuels. The proposed new concept can provide a cleaner and very inexpensive way of bio-hydrogen generation by flexible sheet-like devices.

Received 25th August 2015
Accepted 9th September 2015

DOI: 10.1039/c5ta07112g

www.rsc.org/MaterialsA

Introduction

Molecular hydrogen is produced in a range of living systems, is widespread and is aided by a diverse group of enzymes collectively known as hydrogenase.^{1,2} Hydrogen producing pathways vary widely among prokaryotic and eukaryotic organisms.³ It has been shown that hydrogen is generated by many photoautotrophic organisms⁴ as a part of their metabolism as a by-product of respiration.^{5–7} Some anaerobic bacteria generate hydrogen by degrading carbon substrates *via* anaerobic fermentation, while other organisms, such as green microalgae, generate hydrogen photosynthetically by splitting water and simultaneously producing oxygen.⁸ Hydrogen generation processes aided by microorganisms can involve direct photolysis, indirect photolysis, dark fermentation and photo-fermentation. Here we use the process of photosynthetic

hydrogen production from water by green microalgae which was first discovered by Gaffron and Rubin in 1942.⁹ It is based on conversion of protons to molecular hydrogen by accepting electrons aided by hydrogenase enzyme in the absence of ATP.¹⁰ There are many disadvantages of this process, which is usually based on green microalgae suspended in a batch- or tube-based bioreactor, including scale up challenges, large space requirements and high maintenance costs. The light conversion efficiency of microalgae to hydrogen in commercial installations can be as low as 0.24%,¹¹ hence the requirement to increase the light conversion efficiency for efficient hydrogen evolution.

In this work, we report a new photobioreactor design, based on immobilising green microalgae cultures on a fabric-hydrogel composite (Fig. 1) which addresses some of these challenges. For example, cell immobilisation allows their separation from the circulating culture media. Our design also allows a significant increase of the cell surface density and increases the light utilisation on a per unit mass of cells used. The novel device combines two independent vascular networks for nutrient delivery and hydrogen harvesting adjacent to the layer of immobilised biocomposite. The idea for this design is borrowed from Nature's plant leaves but utilises green microalgae

^aDepartment of Chemistry, University of Hull, Hull, UK. E-mail: v.n.paunov@hull.ac.uk; Tel: +44 (0)1482 465660^bSchool of Engineering, University of Hull, UK^cDepartment of Chemical and Biomolecular Engineering, North Carolina State University, Raleigh, NC 27695, USA

† Electronic supplementary information (ESI) available. See DOI: 10.1039/c5ta07112g



(unicellular) cultures and produces hydrogen in the presence of light and nutrients.

Different techniques have been used to immobilise green microalgae for various applications such as waste water treatment¹² and production of commercially important metabolites.¹³ In other applications, microalgae cells have been immobilised on glass fibres. It has also been demonstrated that the cell immobilisation significantly increased the duration of hydrogen production with rates similar to the suspension culture.¹⁴ However, the irregular deposition and colonisation of the glass fibre surface by the microalgae, has resulted in inefficient distribution of light and nutrients for hydrogen production across the immobilised microalgae population. Algae cells have also been immobilised on fumed silica particles¹⁵ but this did not significantly increase their hydrogen production rate, although it allowed easier separation of the microalgae from the depleted nutrient media for continuous hydrogen production. Other authors reported immobilisation of microalgae by use of latex particles which can trap the cells in thin porous coatings.¹⁶ The hydrogen producing bacteria *Rhodospseudomonas palustris* were immobilised in latex coatings for efficient hydrogen production.¹⁷ This process did show a higher hydrogen production rate but the cells failed to maintain their viability during the drying step for the latex film formation. Microbial cells have routinely been immobilised using hydrogels based on alginate and other media.^{12,13} Heterotrophic^{18,19} and photosynthetic bacteria^{20–23} were successfully immobilised using alginate and other kinds of hydrogels for efficient hydrogen production. The immobilisation of algae cells in hydrogel has many advantages. Hydrogels such as alginate are natural polymers and are not toxic to the algae. The pH of the native hydrogel solutions (as prepared) is about 7 which is favourable for cell growth and division. Alginate hydrogel setting takes place in the presence of Ca^{2+} ions which are biocompatible with the microalgae and do not change the pH in the hydrogel matrix resulting in good cell viability. The hydrogel-like alginate and agar are readily available and can be

produced in large quantities from renewable sources. Microalgae cells have previously been immobilised in alginate in the form of beads,^{24–26} but alginate beads have low light utilisation efficiency. Thin layers of alginate hydrogel have sufficient transparency, however they are mechanically unstable, hence there is need for a substrate to provide mechanical stability to cells entrapped in alginate in the form of biofilms. We solved this problem by using a composite of a specially selected fabric whose pores act as an attachment framework for alginate hydrogel films in which the microalgae cells are entrapped.

We fabricated *C. reinhardtii*-fabric-hydrogel composite materials with adjacent vascular systems in order to enable photosynthesis similar to plant leaves that provide the cells with minerals, nutrients and water for the generation of hydrogen as a by-product. It has been shown previously how microalgae can be coated on microfluidic supports for gas transport and harvesting by-products.^{27,28} Our current study shows that it is possible to immobilise cells using hydrogel on different substrates and then use one network of microfluidic channels to provide these bio-composites with the required nutrients and another one for extraction of desired by-products. The schematic of the concept design of our artificial leaf device is shown in Fig. 1.

We demonstrate that the immobilization of microalgae in a fabric-hydrogel composite allows uniform media distribution whilst maintaining complete anaerobic conditions for the efficient production of hydrogen.

Materials and methods

Materials

Microalgae cultures were grown in Tris-Acetate-Phosphate (TAP) culture medium with an incubation temperature of 30 °C. The culture medium for *C. reinhardtii* consisted of TAP salts (ammonium chloride, NH_4Cl ; magnesium sulphate, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ and calcium chloride $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$), phosphate buffer solution and Hutner's trace elements solution (EDTA disodium salt, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, H_3BO_3 , $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$), all purchased from Sigma-Aldrich, UK. Poly(allylamine hydrochloride) (PAH, M.W. 70 kDa) was also purchased from Sigma-Aldrich. Deionised water produced by a Milli-Q reverse osmosis system (Millipore, UK) was used in all experiments.

Algal strain and growth conditions

The *C. reinhardtii* cc-124 strain was kindly provided by the Flickinger Research Group at North Carolina State University, USA. The cells were grown photoheterotrophically in acetate-rich medium in 100, 250 and 500 mL Erlenmeyer flasks containing 50, 150 and 250 mL of standard Tris-Acetate-Phosphate (TAP + S) medium at pH 7.0.²⁹ The flask was placed in a water jacket connected to a thermostatic water bath maintained at 25–30 °C and placed on a magnetic stirrer with a stirring bar to keep the culture agitated at 500 rpm. The setup was illuminated using two 23 W cool white lights (2800 Lux) providing photosynthetically active radiation of $36.4 \mu\text{E m}^{-2} \text{s}^{-1}$. The cells were grown up to three days and harvested in the late logarithmic

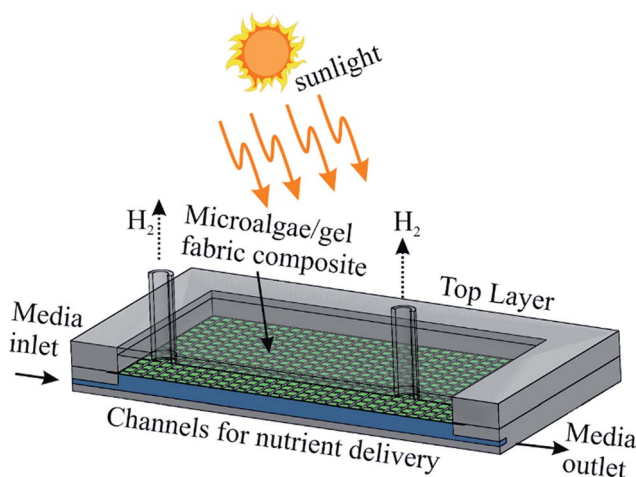


Fig. 1 Conceptual design of the artificial leaf device showing the vascular networks for nutrient delivery to the cells at the bottom and hydrogen extraction from above the *C. reinhardtii*-fabric composite.



growth phase by centrifugation at 3000 rpm for 5 min. All glassware was autoclaved at 121 °C for 20 min before use for cell growth.

Microalgae immobilisation procedure

The procedures for cell and medium preparation and immobilization are schematically shown in Fig. 2. The substrates used for the immobilisation of cells were polyester fabrics purchased from Boyes Stores Ltd (UK) with pore sizes of 1000, 500 and 250 μm , respectively. The fabrics were pre-treated with the cationic polyelectrolyte PAH in order to improve their adhesion properties for the alginate hydrogel. The fabric was first incubated in 1% (w/v) PAH solution in 1 mM NaCl for 1 h. It was then washed several times with 1 mM NaCl solution and dried. The microalgae cells were grown in TAP + S media in aerobic conditions until the culture reached the late log phase. The cells were washed thrice with Milli-Q water by centrifugation at 3000 rpm for 5 min and dispersed in Milli-Q water. The cell suspension was then mixed with aqueous solution of 5% (w/v) alginate (sodium alginate from brown algae, W201502, Sigma

Aldrich, UK). The sodium alginate gel had been prepared using a thermostat water bath set at 95 °C and a homogenizer (Ultra Turrax IKA T25 digital at 11 000 rpm). The gel was completely dissolved in water and sterilized in an autoclave (Prestige Medical 2100) at 121 °C for 20 min. The temperature was lowered to 40 °C in order to mix with the cell suspension. Cells of 1–2 g wet cell weight were dispersed in the aqueous solution and equilibrated at 40 °C for a few minutes before mixing. Next, the aqueous cell suspension was mixed with sterile 5% (w/v) alginate pre-warmed at 40 °C. Following this, the microalgae suspension in alginate solution was poured onto the fabric patch (25 cm \times 10 cm). The gel was uniformly spread across the fabric using a glass rod and allowed to cool down to room temperature as shown in Fig. 2.

The fabric was then sprayed on both sides with 2% (w/v) CaCl_2 aqueous solution at room temperature for further strengthening of the sodium alginate hydrogel layer.

Hydrogen production controls

The *C. reinhardtii* cells were washed thrice with TAP–P–S medium using a centrifuge at 3000 rpm for 5 min and re-suspended in the same medium in the designed bioreactor for hydrogen production. The bioreactor used for hydrogen production process was custom made using borosilicate glass with Rodaviss joints (Sci Labware, UK) which provided a high vacuum seal while holding the glassware without a clamp. The outside of the joint was externally threaded to fit a CG-183 cap and a corresponding CG-305 O-ring to form a non-grease seal. The sampling ports were sealed using the Chromacol 18 mm magnetic screw cap & seal and chlorobutyl septum (Thermo Scientific, UK). The tubing used for the gas collection was hydrogen-proof Tygon tubing (Cole Parmer, UK) with an inner diameter of 0.8 mm and length of 30 cm. A 21 gauge 38 mm Neolus stainless steel needle (Terumo, UK) was used for sampling and gas collection. The flask was sealed and flushed with argon gas for 1 h to remove any residual oxygen in the system and initiate anaerobiosis after which it was exposed to the light source (23 W cool white light, average 50 cm distance from source to photobioreactor surface) with a hydrogen gas collection unit attached to neck of the flask. The reactor was stirred using a magnetic stirrer at a rate of 500 rpm at all times. The gas was collected in an inverted cylinder filled with water on top of a beaker as shown in Fig. 3a.⁸ A photo of the experimental setup is shown in Fig. 3b for suspended *C. reinhardtii* cells and Fig. 3c–d for the sheet of fabric composite of alginate hydrogel immobilised cells placed in the same bioreactor for comparison purposes.

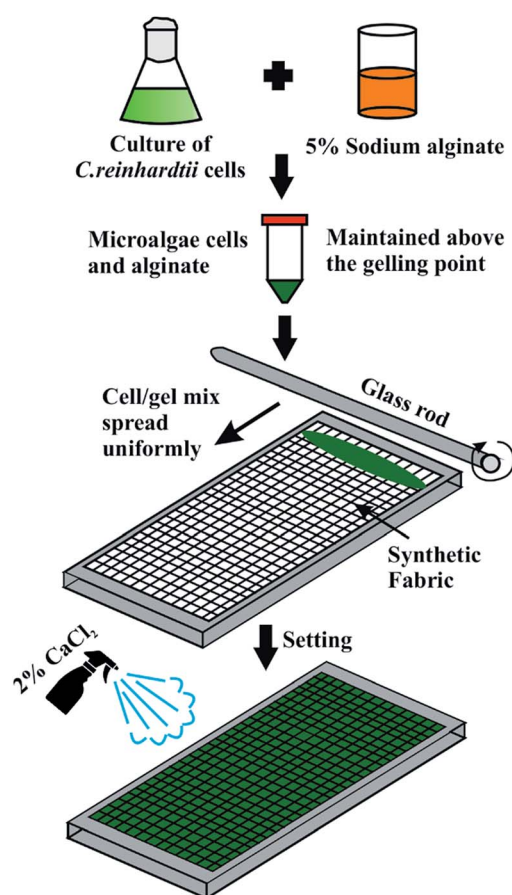


Fig. 2 Schematic of the microalgae immobilisation process. The *C. reinhardtii* cells were grown in suspension culture in aerobic condition and harvested by centrifugation. They were homogenised together with sodium alginate at 5% (w/v) concentration, poured over the synthetic fabric on a glass plate and spread uniformly over the fabric using a sterile glass rod. The alginate films were polymerised by spraying 2% CaCl_2 (w/v) solution.

Design and assembly of the hydrogen generation device

The microfluidic system for nutrient delivery and hydrogen collection was drawn using CAD/CAM software (Solidworks). The designed structures were milled into 5 mm thick sheets of poly(methyl methacrylate) (PMMA) (Kingston Plastics, UK) using a CNC machine (Datron M7). The aluminium metal frame (HE 30 grade) to hold the device was procured from Carters Metals, UK. The schematics in Fig. 4 show the design of the



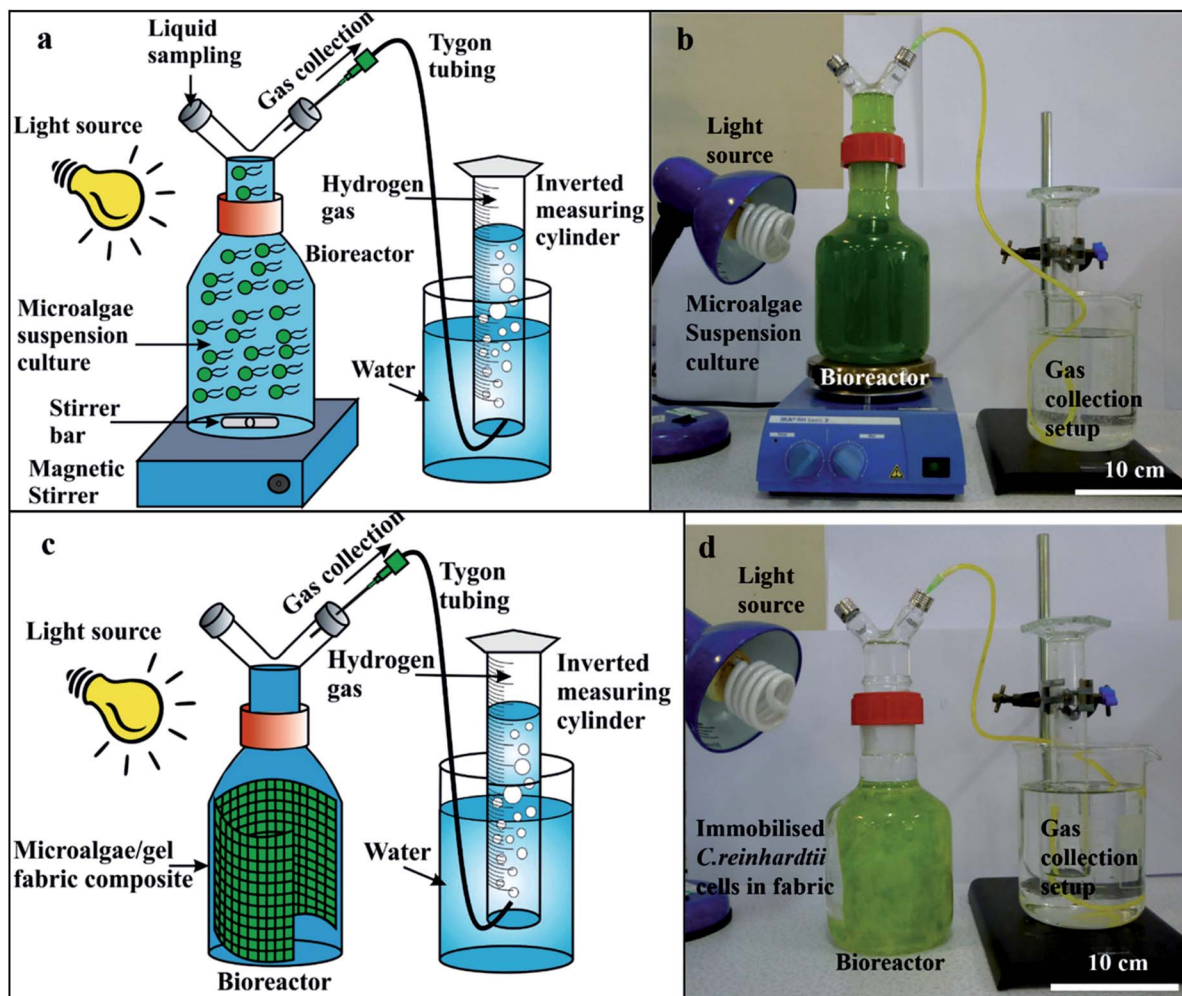


Fig. 3 (a) Schematics and (b) optical photograph of the batch photobioreactor for hydrogen production from *C. reinhardtii* cc-124 strain with an inverted graduated cylinder filled with water in order to measure the volume of hydrogen gas produced. (c) Schematics and (d) photograph of fabric-hydrogel-immobilised *C. reinhardtii* cells suspended in TAP–P–S medium in a batch reactor for bio-hydrogen production. Note that here the *C. reinhardtii* cells are not freely suspended in the batch medium.

'artificial leaf device' for hydrogen production. This design was aimed to allow uniform hydration of the microalgae–hydrogel composite with medium from below and simultaneous extraction of the produced hydrogen from above the composite. The top micro-compartment of the device featured PMMA "islands" as shown in Fig. 4b designed to press onto the fabric-hydrogel composite and secure its position on top of the nutrient delivery channels. The integrated device setup is shown in Fig. 4c where the microalgae culture immobilised on the fabric support was sandwiched in-between an underlying system of microfluidic channels (Fig. 4a) delivering aqueous media free of oxygen and sulphur and a top micro-compartment area (Fig. 4b) from where the released hydrogen was collected. The total length of the bottom and top transparent sheets was 300 mm with the channels positioned within the inner 260 mm. The device cross-sectional assembly of the top and bottom compartments with the sandwiched microalgae–fabric composite is shown in Fig. 5 in more detail. The fabric–hydrogel layer was sealed using a Viton O-ring (RS Components, UK) placed between the top and the bottom vascular system of channels and compressed tightly

using two metal frames: one from the top and the other from the bottom tightened using screws and nuts. The top layer consisted of two outlets: one for the collection of hydrogen and the other for flushing the system with argon to evacuate out any residual air out of the system. The bottom layer consisted of an inlet for the media and an outlet for disposing of the nutrient depleted media after use.

There were two different clamps used to secure the microalgae–fabric composite in its place. The first one was positioned in the underside of the top layer which held the composite between the channels without impeding the flow of media as shown in Fig. 5a. The other clamp was fixed at the edge of the device in the form of a hill and valley lock system where the hill was a part of the bottom layer and the valley to fit the hill perfectly was part of the top layer as shown in Fig. 5b. The fabric was held in-between these two parts in order to stop it from impeding the flow of the media and also to prevent it from filling the top compartment where the produced hydrogen was collected. The total length of the device was similar to that of the bottom and top layers.



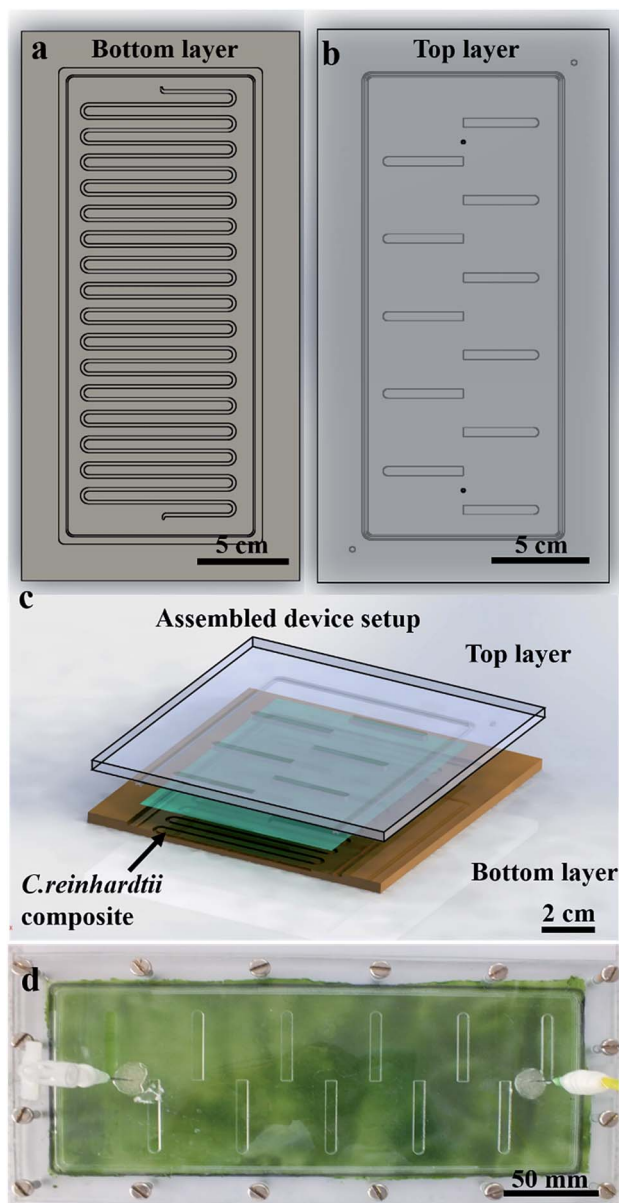


Fig. 4 Schematics of the components of the artificial leaf device (a) bottom layer, (b) top layer, (c) sandwiched *C. reinhardtii*-fabric composite between the top and bottom layer and (d) the photograph of *C. reinhardtii*-alginate fabric composite inside the assembled device.

The bottom channel was pre-filled with TAP-P-S media before placing the *C. reinhardtii* composite on the channels in order to avoid trapping air bubbles inside the channels after incubation.

The device was sealed and initially flushed with argon through the two outlets in the top compartment. The device was illuminated with visible light from the transparent top compartment. The TAP-P-S media was degassed before it was pumped into the device.

Hydrogen production from the 'artificial leaf device'

After harvesting the microalgae cells were washed thrice in TAP-P-S media using the centrifuge at 3000 rpm for 5 min, weighed

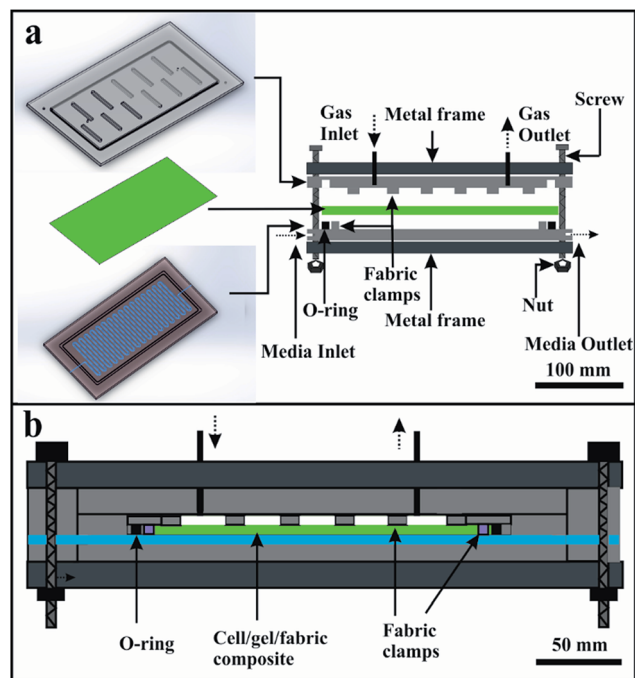


Fig. 5 (a) Expanded view of the 'artificial leaf device' showing the different parts of the assembly: the top layer, *C. reinhardtii* cc-124 composite and the bottom layer. (b) Schematics of the cross-section of the assembled device. The thickness of the compartments and the biocomposite layer is not in scale on this diagram.

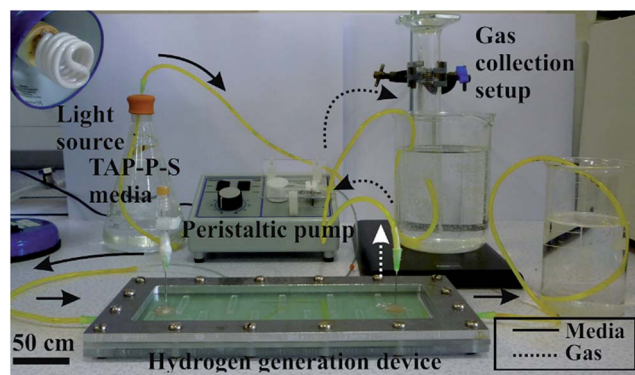


Fig. 6 Photograph of the complete setup of the artificial leaf device for the generation of hydrogen using *C. reinhardtii*-fabric composite.

and re-suspended in Milli-Q water. The cells were homogenized together with alginate solution at a total hydrogel concentration of 5% (w/v) using the Ultra Turrax at 11 000 rpm for 1 min. The polyester fabric was pre-treated with 1% (w/v) PAH solution in 1 mM NaCl to enhance the hydrogel adhesion. Once deposited in the voids of the fabric, the microalgae suspension in the alginate solution was hardened by spraying with 2 wt% aqueous solution of CaCl_2 . The channels of the bottom compartment were pre-filled with TAP-P-S media before the enclosure of the microalgae-fabric composite for hydrogen production. The head space was washed using argon for 1 h. The rate of the flow of media through the device was maintained at 0.26 mL min^{-1} .



The light source used were two 23 W cool white light which emit a photosynthetic proton flux density of $36.4 \mu\text{mol s}^{-1} \text{m}^2$ determined on the surface of the device. The setup of the device for the generation of hydrogen is shown in Fig. 6.

Results and discussion

Microalgae immobilisation on fabric-hydrogel composites

The cell immobilisation procedure described in the methods section was used to entrap the microalgae cells within the fabric-alginate hydrogel composite while maintaining their viability and ability to produce hydrogen. The properties of the support material should satisfy important criteria in maintaining the viability of the immobilised cells. The substrate chosen should have sufficient porosity, durability and longevity, low cost, low toxicity, inertness, ease of availability and stability for the device to function efficiently. Furthermore, the cell deposition procedure should allow a high density of immobilised cells to be retained on the fabric substrate.

We chose poly(ethylene terephthalate) (PET) fabric as the substrate as it is strong, lightweight and resistant to wrinkling. It is also inexpensive and readily available.³⁰ The presence of the fabric in the alginate matrix increased the mechanical stability of the cell-hydrogel composite.

Samples of the microalgae-hydrogel composite deposited on polyester fabric with pore sizes of 1000, 500 and 250 μm are shown in Fig. 7. The stability of the composite depended on the pore size of the fabric. The higher pore size of 1000 and 500 μm retained a smaller amount of cells where some of the voids were completely empty as the cells were only retained on the surface of the fibres of the fabric. However, the fabric with pore size 250 μm was found to be a good compromise and the hydrogel films filling the fabric pores where stable enough and filled with cells (see ESI†). Hence the polyester fabric with a pore size of 250 μm and fibre diameter of 110 μm was chosen for as the substrate for the immobilisation of the *C. reinhardtii* cells in the artificial leaf device for the generation of hydrogen.

The adhesion of the microalgae-hydrogel composite to the fabric in presence of the TAP-P-S media is very important for the integration of the immobilised cells on the fabric hydrogel-composite into the artificial leaf device for hydrogen generation. It is understood that possible separation of the cells from the substrate may potentially cause clogging of the nutrient delivery channels and decrease in the rate of hydrogen production due to the loss of microalgae cells in the process. All hydrophobic fibres like synthetic polyester fibres possess a high negative zeta-potential in water.³¹ This is due to the fact that hydroxyl ions, released due to the dissociation of the water molecule absorb on the hydrophobic surface and this in turn makes the interface negatively charged.

The alginate hydrogel in itself is anionic in nature and its molecular network is negatively charged.³² Hence, in order to increase the adhesion of the hydrogel with the trapped cells to the fabric used as a substrate, the fabric was pre-treated with the cationic polyelectrolyte PAH. We tested the viability of the cells immobilised in the fabric by using FDA after they were deposited on the PAH pre-treated fabric and observed by a fluorescent

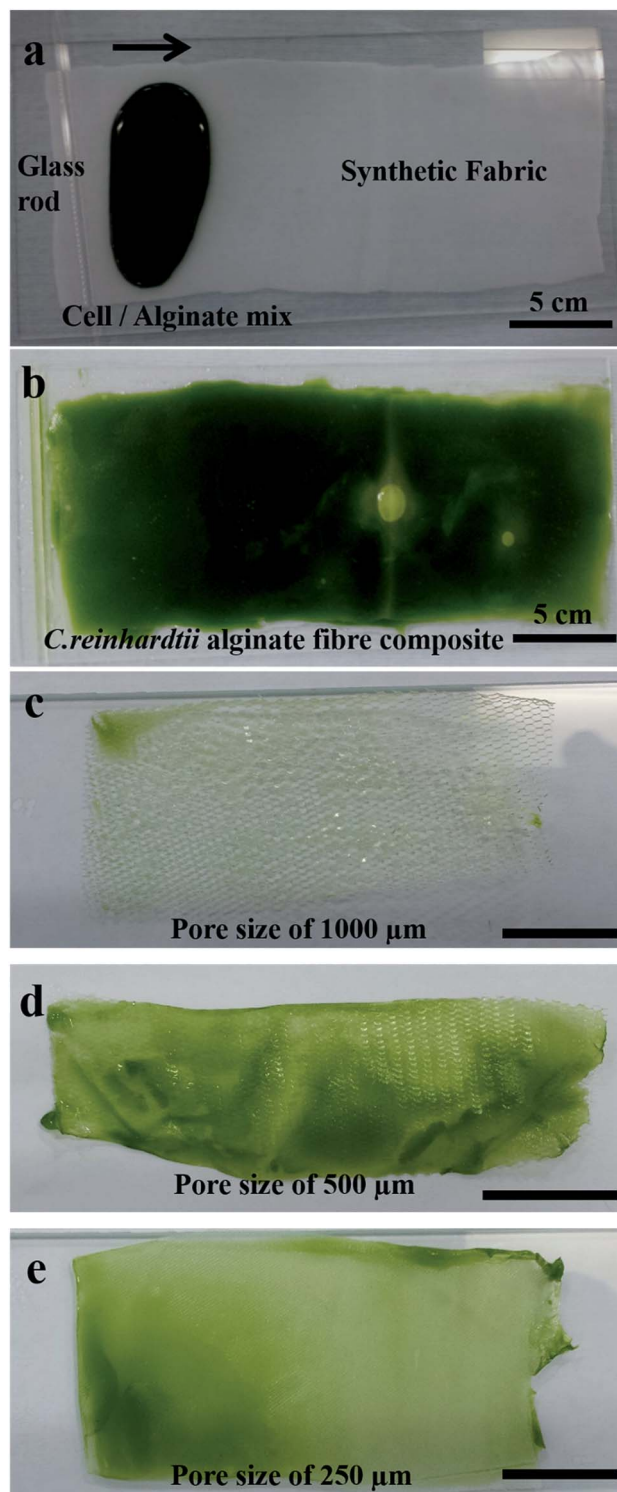


Fig. 7 Photographs of *C. reinhardtii* cells mixed with aqueous 5% (w/v) sodium alginate solution immobilised on different kinds of fabric substrates. (a) The suspension of algae cells in alginate solution was layered on the polyester fabric; (b) cell alginate completely spread over the fabrics; polyester fabrics with a pore size of (c) 1000 μm , (d) 500 μm and (e) 250 μm . All unlabelled scale bars are 10 mm.

microscope. The immobilised cells were found to be highly viable as shown in Fig. 8. The results also show that the microalgae cells were not only present in the alginate hydrogel



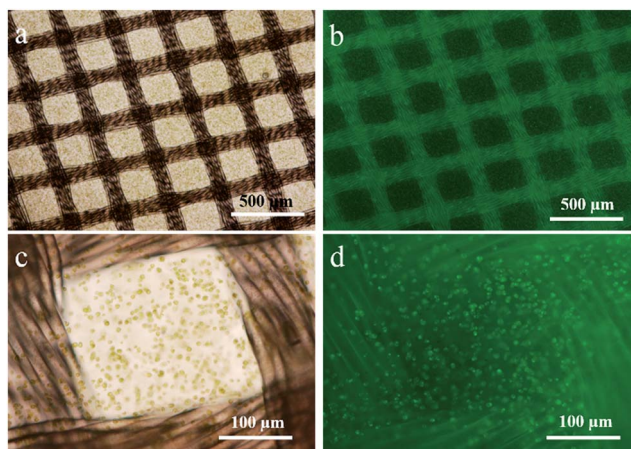


Fig. 8 Microscope images of cells immobilised in a polyester fabric using 5% (w/v) sodium alginate hydrogel. (a) Optical microscopy image of a part of the microalgae biocomposite on the synthetic fabric; (b) fluorescence microscopy image showing the viability of the microalgae cells; (c) optical microscope image of a single pore in the synthetic fabric showing the hydrogel immobilised *C. reinhardtii* cells in the void and (d) a fluorescence microscopy image showing the viability of the cells in the composite embedded in a single pore of the fabric. The cells were treated with FDA after their immobilisation in the fabric for the determination of its viability.

films in the fabric pores but also on the sides of the fibres. These results indicate that the use of sodium alginate can be efficient in the immobilisation of the *C. reinhardtii* cells and is fully biocompatible with them.

Fabrication of the 'artificial leaf device'

Here we discuss the designs and performance of the channel networks for nutrient delivery (bottom layer) and harvesting hydrogen (top layer) in the device. We tested several different designs of the microfluidic system in order to achieve uniform flow of the nutrient media in the device (described in the ESI†). Initially, we tested a design shaped as a leaf for the device and a single channel that ran horizontally and vertically from the inlet to the outlet. In an alternative design the main channel was

interconnected by four other parallel channels. However, in both cases we found that distribution and the flow of media were not uniform through the channels and resulted in formation of stagnant flow regions and the clogging of the device (see ESI†). These designs did not work well for hydrogen generation due to irregular distribution of the media.

The successful design on which we based our working device prototype had a rectangular shape and a single channel of dimensions ranging from 1 mm width and 0.4 mm depth to 2 mm width and 0.8 mm depth. The results obtained from the distribution experiment lead to the use of this rectangular design of the fluidic system for delivering the nutrient media to the *C. reinhardtii* composite in the final prototype of the leaf device for hydrogen production. We also faced a challenge due to the movement of the fabric-hydrogel composite inside the device and the build-up of a high backpressure in the device media inlet. This problem was resolved by the subsequent design as the gap between the channels and islands that were introduced in-between the channels from the top layer in order to hold the fabric-gel composite firmly against the bottom layer of the device.

These changes resulted in much more efficient and uniform distribution of the media and were selected for the final design of the device for hydrogen generation. We present below some of the results with hydrogen production with this device and compare it with the performance of the batch photobioreactors.

Hydrogen generation

The TAP-P-S medium was degassed before it was pumped into the artificial leaf device. The cell-fabric composite was maintained in the device for up to 220 h with an average yield of 37 mL hydrogen gas. The rate of hydrogen production obtained from the device was 1 mL h⁻¹, and similar in magnitude to that of the hydrogen production from suspension culture but with a considerably smaller amount of microalgae cells. The rate of hydrogen produced from the device on per gram of cell basis was about 0.7937 mL h⁻¹ g⁻¹ compared to the batch reactor which was about 0.0817 mL h⁻¹ g⁻¹. The cells were efficiently maintained and used in the device and produced hydrogen. The

Table 1 Amounts, rates of hydrogen gas produced, the efficiency, and the theoretical energy yield by *C. reinhardtii* cells in the form of (i) suspension culture in a batch bioreactor, (ii) immobilised culture in a batch bioreactor and (iii) immobilised culture in our artificial leaf device. The total time of incubation for the batch reactor and the device was between 200–250 hours. All experiments were conducted in triplets

Type of culture and reactor used	Amount of <i>C. reinhardtii</i> cells (g)	Average rate of hydrogen produced (mol s ⁻¹ g ⁻¹)	Highest rate of hydrogen produced (mL h ⁻¹ g ⁻¹)	Highest rate of hydrogen produced (mol h ⁻¹ g ⁻¹)	Surface area (m ²)	Theoretical energy per amount of cells (kJ g ⁻¹)	Efficiency (η _c %) per g of cells
(i) Suspension culture in batch reactor	12.5	4.3 × 10 ⁻¹⁰	0.0817	3.34 × 10 ⁻⁶	0.053	0.061	0.02
(ii) Immobilised culture in batch reactor	1.68	3.1 × 10 ⁻⁹	0.7937	3.24 × 10 ⁻⁵	0.025	0.211	0.38
(iii) Immobilised culture in the 'artificial leaf device'	1.72	2.3 × 10 ⁻⁹	0.5814	2.37 × 10 ⁻⁵	0.025	0.212	0.29



fluidic design was successfully implemented for hydration of the whole cellular composite and supplying them with essential nutrients (minerals) for survival and hydrogen production. We found that a considerably higher amount of energy will be derived from the immobilised culture in the bioreactor as well as the device compared to the suspended culture as shown in Table 1 and Fig. 9. The light energy conversion efficiency η_C showed in Table 1 was calculated using eqn (1):

$$\eta_C = \frac{\Delta G_{H_2}^\circ R_{H_2}}{E_s A} \quad (1)$$

where ΔG° is the standard Gibb's energy of the reaction generating the product H_2 ($237\,200\text{ J mol}^{-1}$ at 298 K), R_{H_2} is the rate of the reaction (mol s^{-1}), E_s is the solar irradiance ($1\ \mu\text{E} = 0.214\text{ J}$, the energy of 1 mol of 560 nm photons) and A is the illuminated area (m^2). The surface area was calculated using the formula for surface area calculation for geometric solids like rectangle in case of the device and cylinder in case of the suspension bioreactor. The theoretical energy produced from hydrogen per gram of cells was calculated using the standard enthalpy of formation for water vapour considering there would be enough oxygen to react with the hydrogen as shown in the reaction ($H_2 + 1/2O_2 \rightarrow H_2O$, $\Delta H_f^\circ = -241.81\text{ kJ mol}^{-1}$) and eqn (2).

$$\text{Theoretical energy (kJ)} = \Delta H_f^\circ (\text{kJ mol}^{-1}) \times \text{moles of } H_2 (\text{mol}) \quad (2)$$

The rate of hydrogen production obtained from the device was much higher compared to that of the suspension culture in the photobioreactor on a per gram cell basis. The efficiency data

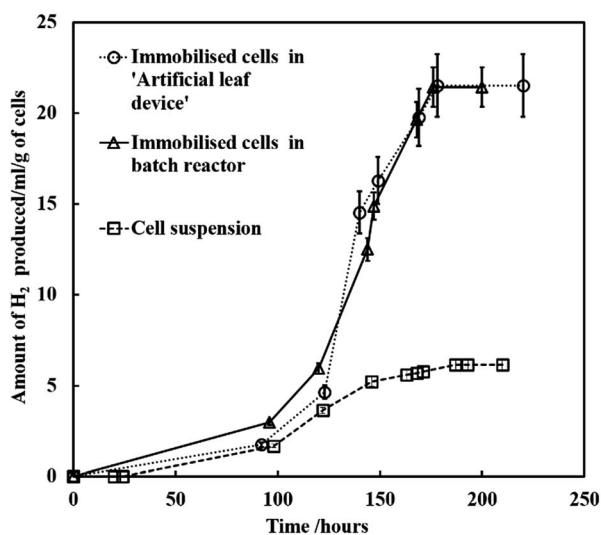


Fig. 9 The volume of the produced H_2 gas (in mL) as a function of the incubation time per gram of suspended and immobilised *C. reinhardtii* cells in a 1 litre batch photobioreactor (dashed line and solid line) and the artificial leaf device (dotted line) in anaerobic condition suspended in TAP–P–S media in a system pre-flushed with argon. The three systems were illuminated with the same light source and media conditions.

of the hydrogen production system (per gram of cells) are shown in Table 1. It is clearly evident that immobilised cell systems are superior compared to the cell suspension system for hydrogen production. The immobilised microalgae system has the additional advantage that it allows regeneration of the trapped microalgae by switching the nutrient depleted media with growth nutrient media. These results showed that immobilised microalgae cultures in a microfluidic system as our artificial leaf device can be much more efficient in hydrogen generation. Previously, the systems used for microalgae production of hydrogen showed efficiencies of 0.24% (suspension culture)¹¹ and 0.36% (immobilised on glass fibres)³³ in a photobioreactor. The data in Table 1 show that we have attained similar efficiencies with immobilised cell culture.

Conclusions

We developed an artificial leaf device based on immobilised microalgae culture. The device is based on a fabric–hydrogel–microalgae biocomposite sandwiched between two adjacent systems of channels for nutrient delivery (bottom layer) and harvesting hydrogen (top layer). The device was designed with the main aim of uniform media distribution and complete anaerobic conditions. Both of these aims were achieved, resulting in the efficient production of hydrogen from the *C. reinhardtii* composite. We produced stable fabric-based composites of *C. reinhardtii* (microalgae) cells using alginate hydrogel trapping and polyelectrolyte mediated adhesion of the hydrogel onto the fabric. This allowed us to control and optimise the exposure of the immobilised cells to light and increase the hydrogen production efficiency. The microalgae immobilisation also allowed us to separate the cells from the liquid media phase, which considerably increased the cell density and hence allowed higher light utilisation on a per area basis. The fabric-based composite of hydrogel-immobilised cells was efficiently integrated in a microfluidic device reminiscent of an artificial leaf. We resolved the challenge to maintain the viability of the immobilised microalgae cells in the prepared hydrogel–fabric composites during the immobilisation procedures and the hydrogen production regime.

The highest rate of hydrogen production obtained from the device was 1 mL h^{-1} similar to that of the immobilised culture hydrogen production in the photobioreactor but with one order of magnitude smaller amount of cells in case of the immobilised culture. The rate, amount and the theoretical energy produced from hydrogen on a per gram basis of cell showed that the immobilisation of algae had a considerable effect on hydrogen production; about 20 times more hydrogen was produced in the immobilised microalgae culture compared to the suspension culture in a batch reactor.

The development of the hydrogen producing artificial leaf device containing immobilised microalgae cells and its superior efficiency to hydrogen production from suspension culture in a photobioreactor is to our knowledge reported here for the first time. We believe that the hydrogen production system from immobilised culture of microalgae cells has these major advantages compared to hydrogen production from suspension



culture. This system of immobilised cell will be easy to scale up and also would require less amount of space compared to the scaled up suspension culture in bioreactors as the fabric-based algae composite can in principle be stacked up in multilayers. This could potentially lower the cost required for the maintenance of the large scale bioreactor. The microalgae immobilisation in planar substrates would result in the separation of cells from the liquid media phase, which can considerably increase the cell density and hence allow higher light utilisation on a per device surface area basis. The light conversion efficiency for hydrogen production of the immobilised algae cells in the bioreactor and the artificial leaf device is higher due to the increased hydrogen production in case of the immobilised cells. The immobilisation of cells in the artificial leaf device also provided the opportunity for the continuous hydrogen production. These results provide a proof of concept that using immobilised microalgae cultures in the artificial leaf device can be more efficient way for bio-hydrogen generation.

Further outlook

The other disadvantage of the suspended cells in liquid media is the difficulty to recycle the batch system between the process of sulphur deprivation and sulphur re-addition for continuous hydrogen production, since this process needs large energy input for the required pumping and centrifugation step for the separation of the algae cells from the sulphur-depleted media to be regenerated in TAP + P + S media. The immobilised cells in the artificial leaf device can be easily cycled between the sulphur depletion phase and the sulphur repletion condition by pumping the desired media through the channels and can potentially open the way to a simpler, cheaper and quicker method for continuous hydrogen production. The *C. reinhardtii* cells immobilised in the system may produce hydrogen in anaerobic conditions and cells can be regenerated by pumping in TAP + P + S media in aerobic conditions. The hydrogen gas collected can be used further in an adjacent fuel cell. A further improvement could be the use of biodegradable fabrics for

immobilisation which would increase the sustainability of the process. The residual algal biomass at the end of the device life-cycle could be fermented by cyanobacteria for further hydrogen generation or used as animal feed as shown in Fig. 10.

There is a huge scope for the further development of the idea for the immobilisation of the photosynthesising cells in artificial leaf devices for hydrogen production. One of the most important feature of these devices is that they can be made in the form of flexible, foldable and inexpensive sheets, bound by polymer films. These sheet-like hydrogen producing devices can be deployed in many areas, which are presently unsuitable for larger and more expensive photobioreactors.^{35,36} There are many different kinds of microorganisms which can utilise light for hydrogen production and which can be tested with this system for more efficient light utilisation and thereby hydrogen production. Recently, *C. reinhardtii* cells were genetically modified and the hydrogen production was increased five folds.³⁴ This microalgae composite can be integrated in the device for more superior hydrogen production.

Acknowledgements

We are very grateful for the sample of *C. reinhardtii* cc-124 strain which was kindly donated to us by Professor Michael Flickinger at North Carolina State University and the advice by Dr Jessica Jenkins at Flickinger's Research group on the media and growth conditions for this algal culture. The cell cultures were produced with funding from US NSF CBET 1510072.

References

- 1 S. P. J. Albracht, *Biochim. Biophys. Acta, Bioenerg.*, 1994, **1188**, 167–204.
- 2 J. W. Peters, *Curr. Opin. Struct. Biol.*, 1999, **9**, 670–676.
- 3 V. A. Boichenko, E. Greenbaum and M. Seibert, in *Molecular to Global Photosynthesis*, 2004, pp. 397–451.
- 4 J. Appel and R. Schulz, *J. Photochem. Photobiol., B*, 1998, **47**, 1–11.
- 5 P. F. Weaver, S. Lien and M. Seibert, *Sol. Energy*, 1980, **24**, 3–45.
- 6 J. Meyer and J. Gagnon, *Biochemistry*, 1991, **30**, 9697–9704.
- 7 G. Voordouw, J. D. Strang and F. R. Wilson, *J. Bacteriol.*, 1989, **171**, 3881–3889.
- 8 A. Melis, L. Zhang, M. Forestier, M. L. Ghirardi and M. Seibert, *Plant Physiol.*, 2000, **122**, 127–136.
- 9 H. Gaffron and J. Rubin, *J. Gen. Physiol.*, 1942, **26**, 219–240.
- 10 V. A. Boichenko and P. Hoffmann, *Photosynthetica*, 1994, **30**, 527–552.
- 11 M. L. Ghirardi, *Indian J. Biochem. Biophys.*, 2006, **43**, 201–210.
- 12 N. Mallick, *BioMetals*, 2002, **15**, 377–390.
- 13 N. Mallick, *Immobilization of microalgae*, Humana Press Inc, Totowa, USA, 2006.
- 14 T. V. Laurinavichene, A. S. Fedorov, M. L. Ghirardi, M. Seibert and A. A. Tsygankov, *Int. J. Hydrogen Energy*, 2006, **31**, 659–667.
- 15 J. J. Hahn, M. L. Ghirardi and W. A. Jacoby, *Biochem. Eng. J.*, 2007, **37**, 75–79.

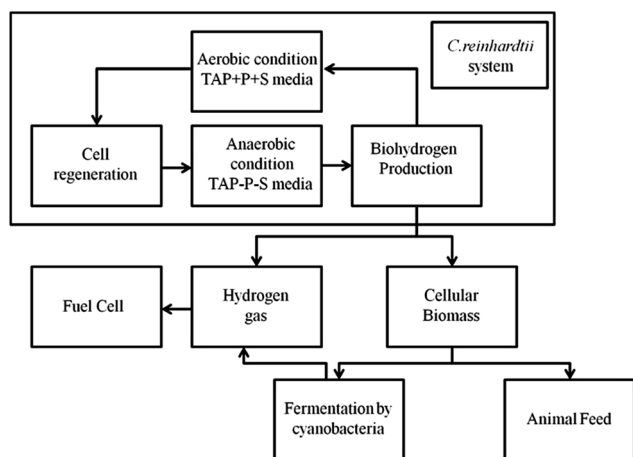


Fig. 10 Schematic representation of the life-cycle of a bio hydrogen generating system based on immobilised *C. reinhardtii*.



- 16 M. C. Flickinger, J. L. Schottel, D. R. Bond, A. Aksan and L. E. Scriven, *Biotechnol. Prog.*, 2007, **23**, 2–17.
- 17 J. L. Gosse, B. J. Engel, F. E. Rey, C. S. Harwood, L. E. Scriven and M. C. Flickinger, *Biotechnol. Prog.*, 2007, **23**, 124–130.
- 18 A. Kumar, S. R. Jain, C. B. Sharma, A. P. Joshi and V. C. Kalia, *World J. Microbiol. Biotechnol.*, 1995, **11**, 156–159.
- 19 S. Y. Wu, C. N. Lin, J. S. Chang, K. S. Lee and P. J. Lin, *Biotechnol. Prog.*, 2002, **18**, 921–926.
- 20 K. Sasikala, C. V. Ramana and P. R. Rao, *Int. J. Hydrogen Energy*, 1992, **17**, 23–27.
- 21 M. Vincenzini, R. Materassi, M. R. Tredici and G. Florenzano, *Int. J. Hydrogen Energy*, 1982, **7**, 725–728.
- 22 M. Vincenzini, R. Materassi, M. R. Tredici and G. Florenzano, *Int. J. Hydrogen Energy*, 1982, **7**, 231–236.
- 23 N. Rashid, W. Song, J. Park, H. F. Jin and K. Lee, *J. Ind. Eng. Chem.*, 2009, **15**, 498–503.
- 24 A. L. Dainty, K. H. Goulding, P. K. Robinson, I. Simpkins and M. D. Trevan, *Biotechnol. Bioeng.*, 1986, **28**, 210–216.
- 25 I. Garbayo, E. Forjan, A. Salguero, M. Cuaresma, J. M. Vega and C. Vilchez, *Biotechnol. Lett.*, 2005, **27**, 265–267.
- 26 C. Vilchez, I. Garbayo, E. Markvicheva, F. Galvan and R. Leon, *Bioresour. Technol.*, 2001, **78**, 55–61.
- 27 H. J. Koo and O. D. Velev, *Sci. Rep.*, 2013, **3**(2357), 1–6.
- 28 A. Magnuson, M. Anderlund, O. Johansson, P. Lindblad, R. Lomoth, T. Polivka, S. Ott, K. Stensjö, S. Styring, V. Sundström and L. Hammarström, *Acc. Chem. Res.*, 2009, **42**, 1899–1909.
- 29 R. A. Andersen, *Algal culturing techniques*, Elsevier/Academic Press, Burlington, Mass, 2005.
- 30 A. J. East, in *Synthetic Fibres*, ed. J. E. McIntyre, Woodhead Publishing, 2005, pp. 95–166.
- 31 H. T. Lokhande, N. R. Mody, K. N. Rao and M. H. Rao, *J. Appl. Polym. Sci.*, 1979, **23**, 2139–2146.
- 32 L. Jiang, Y. Lu, X. Liu, H. Tu, J. Zhang, X. Shi, H. Deng and Y. Du, *Carbohydr. Polym.*, 2015, **121**, 428–435.
- 33 T. V. Laurinavichene, S. N. Kosourov, M. L. Ghirardi, M. Seibert and A. A. Tsygankov, *J. Biotechnol.*, 2008, **134**, 275–277.
- 34 S. Rumpel, J. F. Siebel, C. Fares, J. Duan, E. Reijerse, T. Happe, W. Lubitz and M. Winkler, *Energy Environ. Sci.*, 2014, **7**, 3296–3301.
- 35 J. S. Jenkins, M. C. Flickinger and O. D. Velev, *Materials*, 2013, **6**, 1803–1825.
- 36 H.-J. Koo and O. D. Velev, *J. Mater. Chem. A*, 2013, **1**, 11106–11110.

