Electronic Supplementary Information

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

Anupam A. K. Das[a], M. M. Nasr Esfahani[b], Orlin D. Velev[c], Nicole Pamme[a] and Vesselin N. Paunov*[a]

[a] *Department of Chemistry, University of Hull, Hull, United Kingdom,, Tel: +44 (0)1482 465660; E-mail: v.n.paunov@hull.ac.uk*

[b] *Department of Engineering, University of Hull, United Kingdom.*

[c] *Department of Chemical and Biomolecular Engineering North Carolina State University, Raleigh, NC 27695, USA.*

Immobilisation of *C. reinhardtii* cells onto fabric substrates

(a) Fabric requirements

Here we consider the important factors for successful immobilisation of microalgae for hydrogen generation. The support matrix for the immobilisation of living cells is one of the most important factor in maintaining the cells viability and thereby the efficient production and recovery of the produced hydrogen from the microfluidic system. In addition to the requirement for the support material to be biocompatible with the algal cells, its mechanical and chemical stability, flow properties, durability, inertness and lack of toxicity, there are also economic factors like low fabrication cost, commercial availability and recycling costs.

b) Cell immobilisation procedure

The cell immobilisation procedure is required to allow high concentration of cells on the substrate without allowing the detachment of cells from the support matrix under flow condition or as a result of further cell growth. The immobilisation process should also be fast, cheap and allow the viability of the cells to be maintained.

The polyester fabrics (poly (ethylene terephthalate), PET) is very strong, light weight and resistant to wrinkling. We chose polyester fabrics as substrates for our biocomposites as they are inexpensive, readily available from a range of manufacturers and suppliers and meet all the compatibility requirements. However, we found that upon deposition of the hydrogel with the

cells on the fabric, it had poor adhesion to the hydrogel without any pre-treatment. We solved this problem by pre-treating the PET fabric with the cationic polyelectrolyte PAH followed by washing of the excess of PAH. This step significantly improved the hydrogel adhesion, as shown in the following section. Another important factor is maintaining sterile environment during the biocomposite preparation which does not allow microbial contamination of the hydrogel or the prepared biocomposite before it is integrated into the hydrogen generation device.

c) Importance of the fabric pore size

The stability of the hydrogel-microalgae composite also depends on the pore size of the fabric, as usually a film of hydrogel solution containing the cells is supported by the fibres forming the pores in the fabric material. The wettability of the PET fabric by the hydrogel solution and the hydrogel film stability determines the success of the cell immobilisation on the supporting fabric. We found that PET fabrics of pore size of 1000 μm and retains smaller amount of cells as shown in Figure S1 (b). In this case the fabric pores were void due to low stability of the hydrogel films which led to retention of algal cells only on the fibres of the fabric. In contrary, the fabric of pore size 250 μm and fibre diameter of 110 μm formed stable hydrogel films and as shown in Figure S1 (a) which contained higher cell density per area of fabric. This type of PET fabric was selected as the substrate for the immobilisation of the *C.reinhardtii* cells to be incorporated in the artificial leaf device for the generation of hydrogen. The hydrogel-algae coating was strengthened by spraying the fabric-hydrogel composite with 2 wt% $CaCl_2$ solution which led to instant gelation of the alginate.

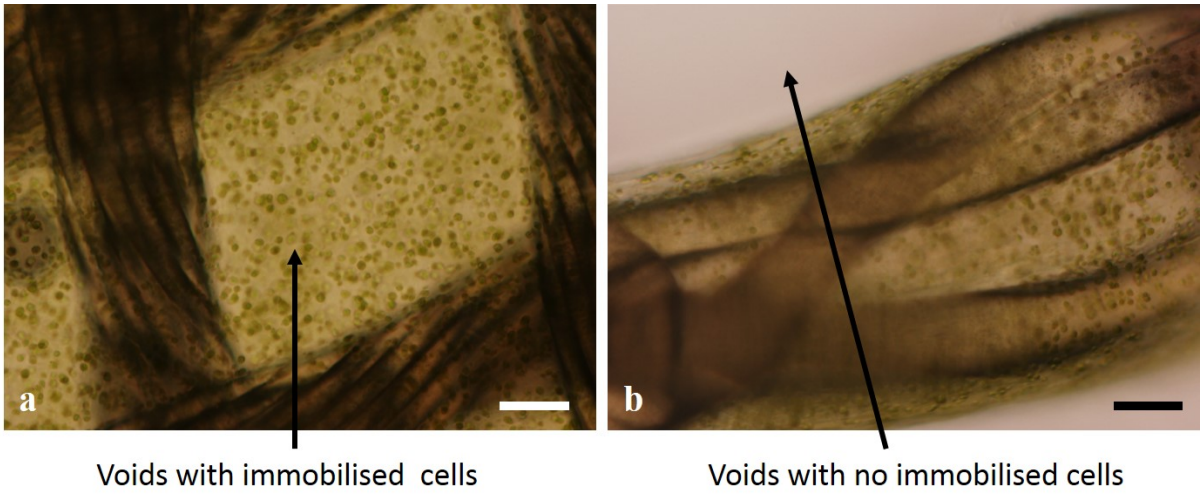

Figure S1. Optical microscope images of *C. reinhardtii* cells mixed with sodium alginate solution and immobilised in a polyester fabrics of pore sizes: (a) 250 µm and (b) 1000 µm. Both scale bars are 50 µm.

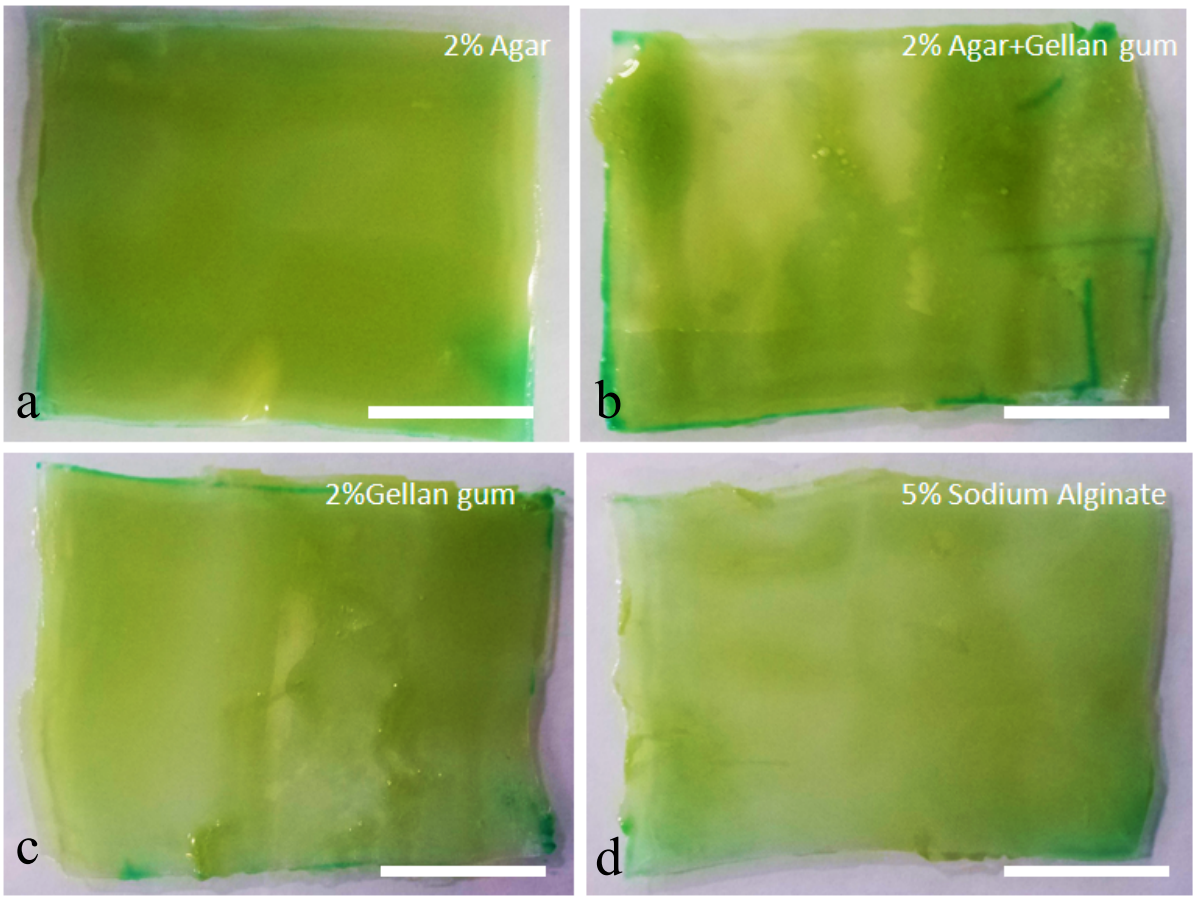

Figure S2 Optical photograph of *C. reinhardtii* cells immobilised on a synthetic (PET) fabric using (a) 2 % (w/v) agar, (b) 2 % (w/v) agar + gellan gum, (c) 2 % (w/v) gellan gum and (d) 5% (w/v) sodium alginate aqueous solutions. All scale bars are 3 cm.

We also experimented with other immobilising agents to incorporate the microalgae cells within the fabric material. We tried several different gelling polysaccharides like agar, gellan gum and sodium alginate. The microalgae cell-hydrogel mixture was prepared according to the method described in details in the materials and method section and layered uniformly using a Pasteur pipette on the fabric as shown in Figure S2.

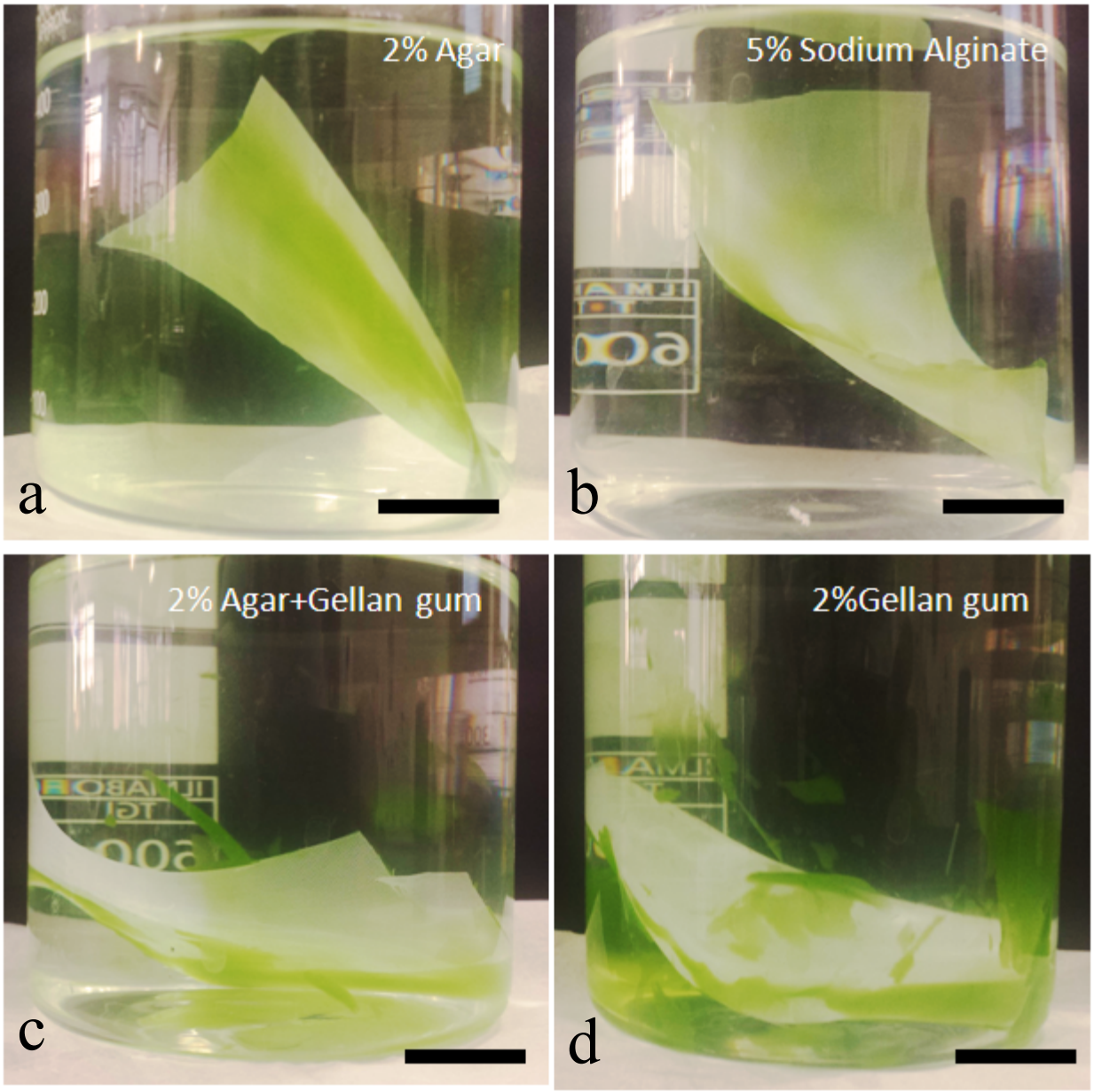

Figure S3. Photograph of the biocomposite of *C. reinhardtii* cells immobilised using (a) 2% (w/v) agar, (b) 5% (w/v) sodium alginate, (c) 2% (w/v) of 50:50 agar + gellan gum and (d) 2% (w/v) gellan gum on a synthetic (PET) fabric suspended in TAP-P-S media after 1 hours. All scale bars are 5 cm.

Although it was easy to layer the agar and the sodium alginate based cell suspensions on to the fabric, for gellan gum based solutions we encountered challenges due to faster gelation of the solution resulting in a non-uniform distribution of the microalgae cells in the hydrogel mixture as it was deposited on the polyester fabric (see Figure S2 (c)). The adhesion of the microalgae-hydrogel mixture to the fabric in presence of the TAP-P-S media was quite 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. The cell-hydrogel loaded fabric was incubated in the TAP-P-S media in order to determine the stability of the composite for 1 hour. The fabric loaded with microalgae with sodium alginate hydrogels showed sufficient stability as shown in Figure S3 (a) and (b) after 1 hour of incubation.

All hydrophobic fibres like synthetic polyester fibres possess a high negative zeta-potential.[1] The sodium alginate gel in itself is anionic in nature and its molecular network in solution is negatively charged.[2] In order to increase the adhesion of the hydrogel matrix to the fabric support used as a substrate with sodium alginate as an immobilising agent, the fabric was pre-treated using PAH, a cationic polyelectrolyte, using the method described in the materials and method section. This significantly improved the adhesion of the fabric to the sodium alginate hydrogels and the trapped microalgae into the fabric substrate. In the case of gellan gum and mixtures of gellan gum and agar, the hydrogel-cells composite had very poor adhesion to the fabric and peeled off in TAP media as evident from Figure S3 (c) and (d), respectively. Hence the use of gellan gum as a fabric immobilising agent for *C. reinhardtii* cells in the artificial leaf device would be inefficient and may cause problems with channel clogging with debris from eventual detachment of the gellan hydrogel from the fabric. Therefore, we chose alginate hydrogels for immobilisation of the *C. reinhardtii* cells and the subsequent hydrogen production from the immobilised cultures.

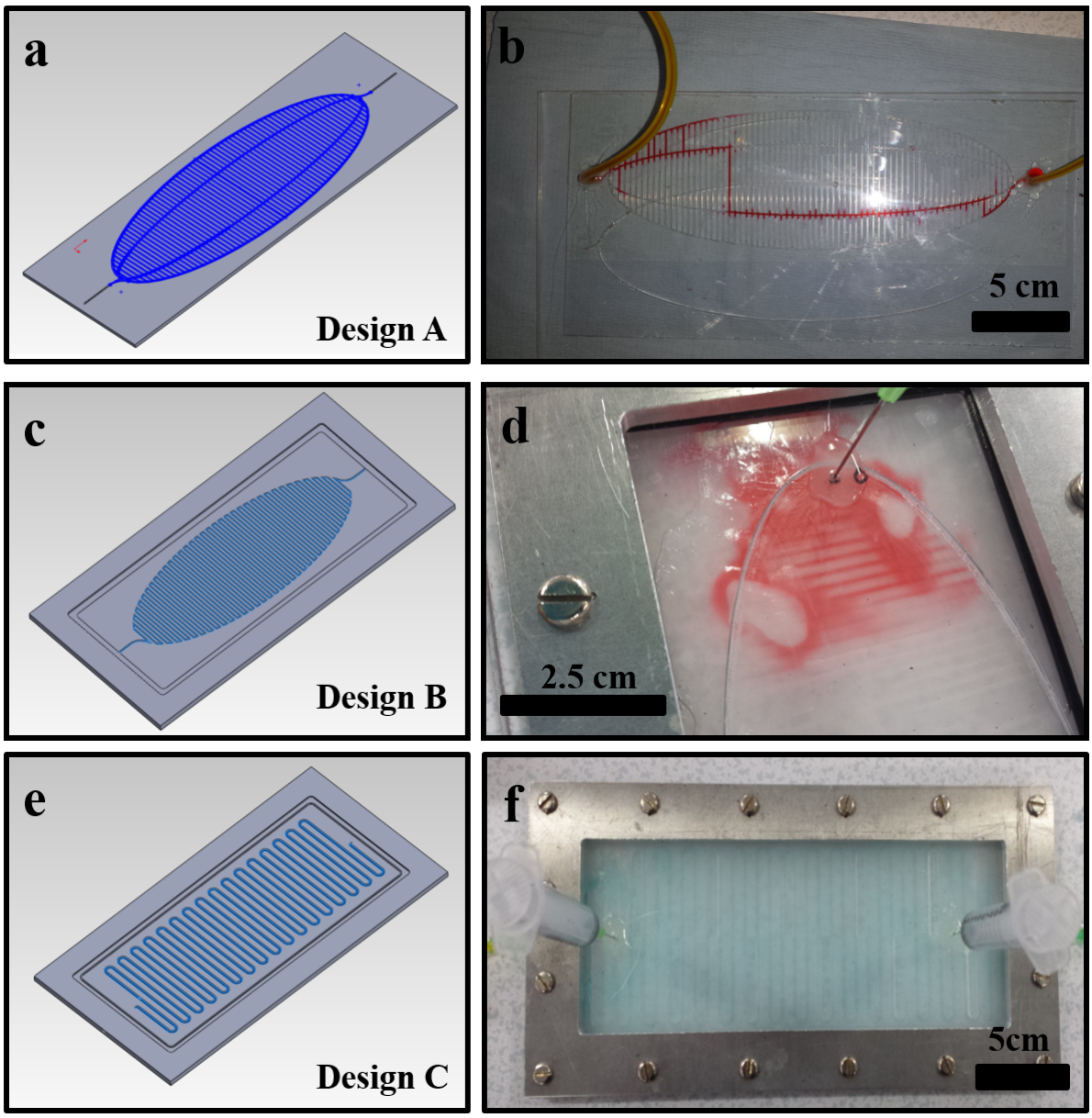

Figure S4. Schematics and photographs of various designs tested for the uniform distribution of nutrient media in the 'artificial leaf device' for hydrogen generation. The coloured dye solution was used to visualise shows the movement of the media in the device. The images show a range of unsuccessful designs (a)-(d) and the successful design (e)-(f).

This section deals with various designs of the fluidic system used for the uniform flow and distribution of culture media in the hydrogen production device. The main aim was to uniformly hydrate the biocomposite composed of *C. reinhardtii* cell with sulphur and phosphate deprived TAP media for their efficient production of hydrogen.

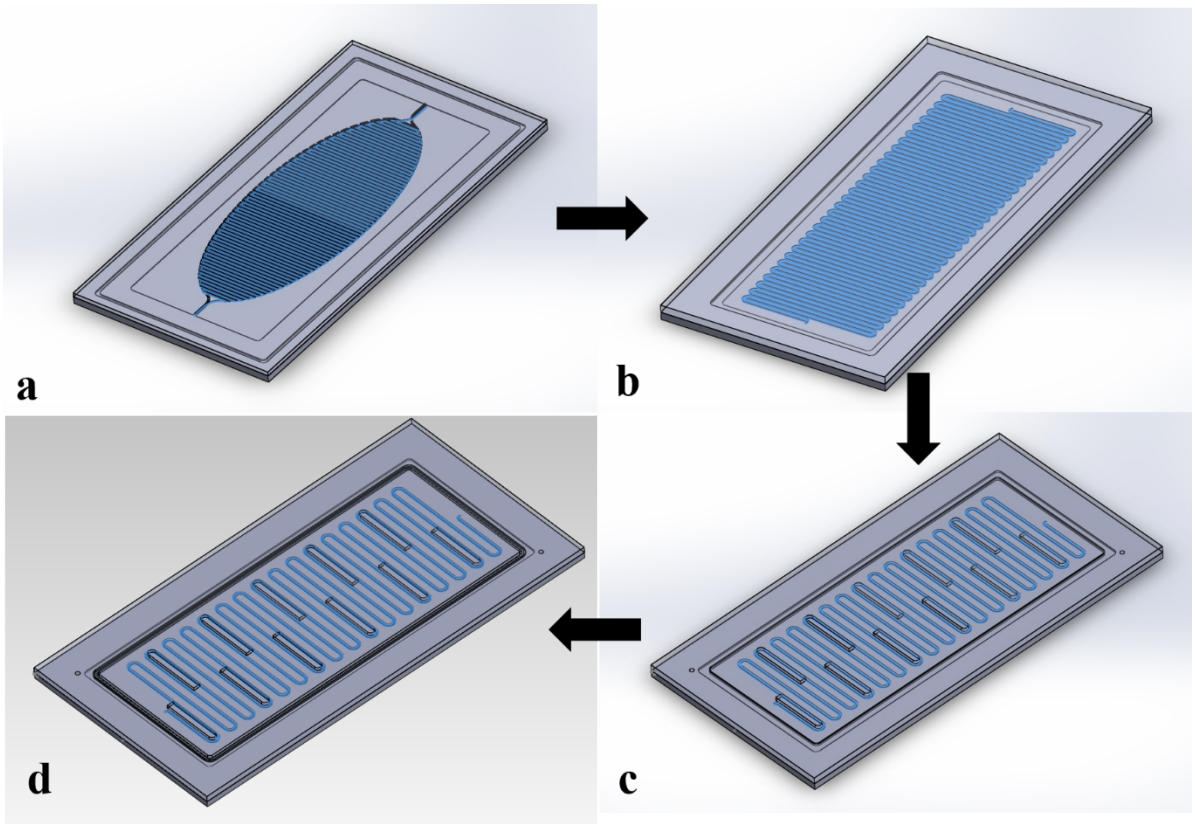

Figure S5. Schematics of various designs tested for the 'artificial leaf device' for efficient hydrogen generation. The arrows shows the design evolution of the device from (a) preliminary leaf shaped design to (b) rectangular design ad (c) rectangular design with a modified top layer consisting of islands in between the channels. (d) The final rectangular design with the fabric clamp surrounding the channels. The working prototype was based on design (d).

Design of the artificial leaf device fluidic systems for culture media delivery

The preliminary idea was to have a design for the device shaped as a leaf as shown in Figure S4 (a) and (c). In both cases the channel width was initially 1 mm and the depth was 0.4 mm. In the schematic shown in Figure S4 (c), a single channel ran horizontally and vertically from the inlet to the outlet whereas in Figure S4 (a), it was interconnected by three other parallel channels. However, the tests with a media stained a dye showed that the flow and media distribution were not uniform and resulted in the clogging of the device, as shown in Figure S4 (b) and (d).. The rate of the flow of the stained media was maintained at 0.26 ml min^{-1} for all cases. This design was unsuited for the hydrogen generation device due to irregular distribution

of the media, hence we experimented with another design as shown in Figure S4 (e) which had a rectangular shape rather than leaf-shaped. The channel width was increased to 2 mm and the depth to 0.8 mm. Similar experimental flow conditions were applied to determine the movement and distribution of the media through the device. As evident from Figure S4 (f) the distribution and media flow were also uniform in the channels for the whole length of the device. The results obtained from the media distribution experiment lead to the use of this particular design of the fluidic system for delivering the nutrient media to the *C. reinhardtii* composite in the final leaf device for hydrogen production. The designs showed in Figure S5 were integrated in the artificial leaf device along with the fabric-hydrogel biocomposite as shown in Figure S6. The leaf shaped design was abandoned due to the ineffective distribution of the media as showed in Figure S4 (d) in the parallel channels of unequal length. We therefore considered rectangular designs to circumvent this problem. There were three different rectangular designs tested as shown in the Figure S5 (b), (c) and (d), respectively. We found the densely-packed channels considered in the design on Figure S5 (b) ineffective when integrated in the final device the distribution of media due to the back pressure and the high hydrodynamic resistance of such device. This caused shifting of the fabric-hydrogel composite inside the device due to the build-up of pressure during the distribution of the culture media throughout the channel. In the subsequent design shown in Figure S5 (c), the gap between the channels was increased compared with the previous design and islands were introduced in between the channels from the top layer in order to hold the fabric gel composite firmly inside the artificial leaf device. These changes improved the distribution of the media in the device as shown in Figure S6 (b) but few problems of clogging of the channels were still evident. Further modification to the device was done by introducing of fabric clamps around the channels to prevent the clogging from parts of the fabric-hydrogel biocomposite. The schematic of this design is shown in Figure S5 (d) and the tests showed efficient and uniform distribution of the media through the device as shown in Figure S6 (c). This successful design of the device was selected for the working prototype for hydrogen production.

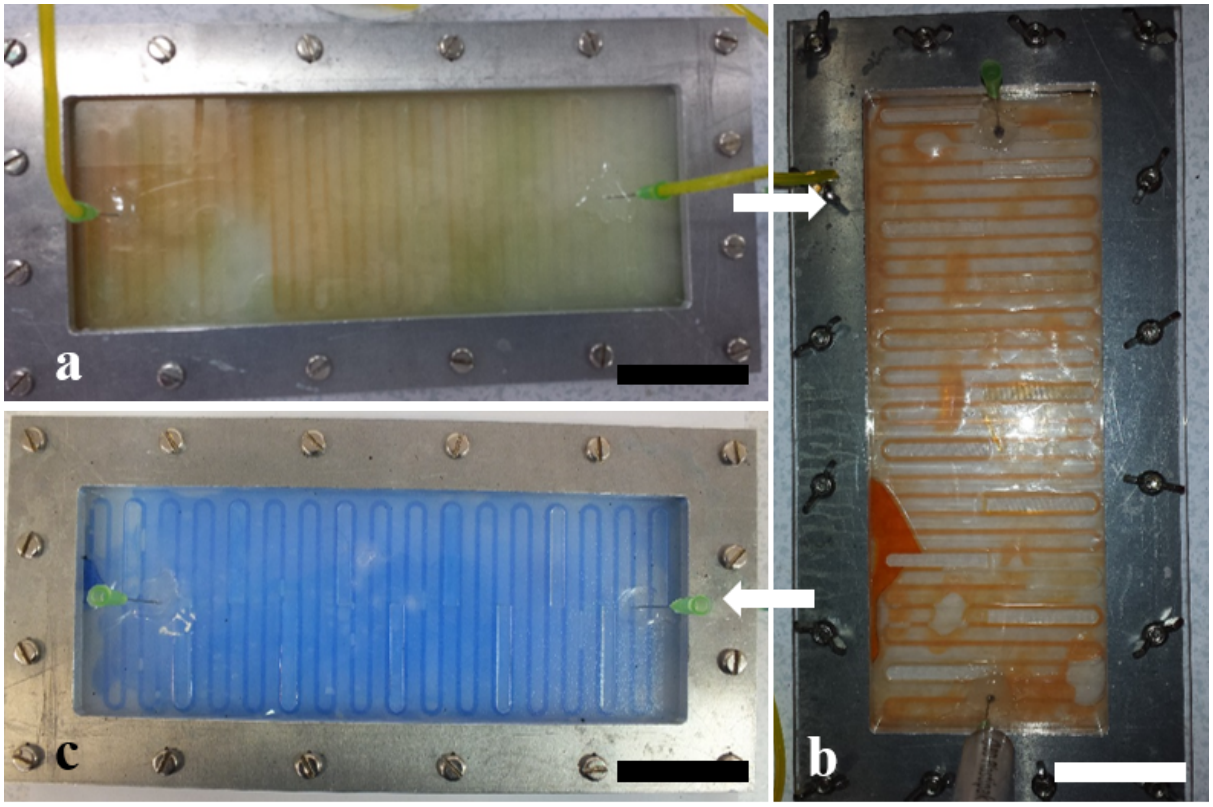

Figure S6. Photographs of various tested unsuccessful and the final working designs of the 'artificial leaf device' for hydrogen generation from immobilised microalgae cultures. The arrows shows the evolution of the overall design of the artificial leaf device from (a) rectangular design to (b) rectangular design with a modified top layer consisting of islands in between the channels and to (c) the final rectangular design with the fabric clamp surrounding the channels. All scale bars are 5 cm.

The dimensions of the channel integrated in the bottom layer are shown in Figure S7. There were two different clamps used to hold the fabric-hydrogel biocomposite containing the immobilised microalgae in its place. The first one was present in the underside of the top layer which holds the composite between the channels without impeding the flow of media as shown in Figure S7 (c). The other clamp was positioned 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 as a part of the top layer (see Figure S7 (b)). The fabric composite sealed the bottom layer channels from above and was held in between these two parts in order to stop it from impeding the flow of media and also to prevent it from shifting into the top compartment for collection of hydrogen. The total length of the device was similar to that of the bottom and top layers.

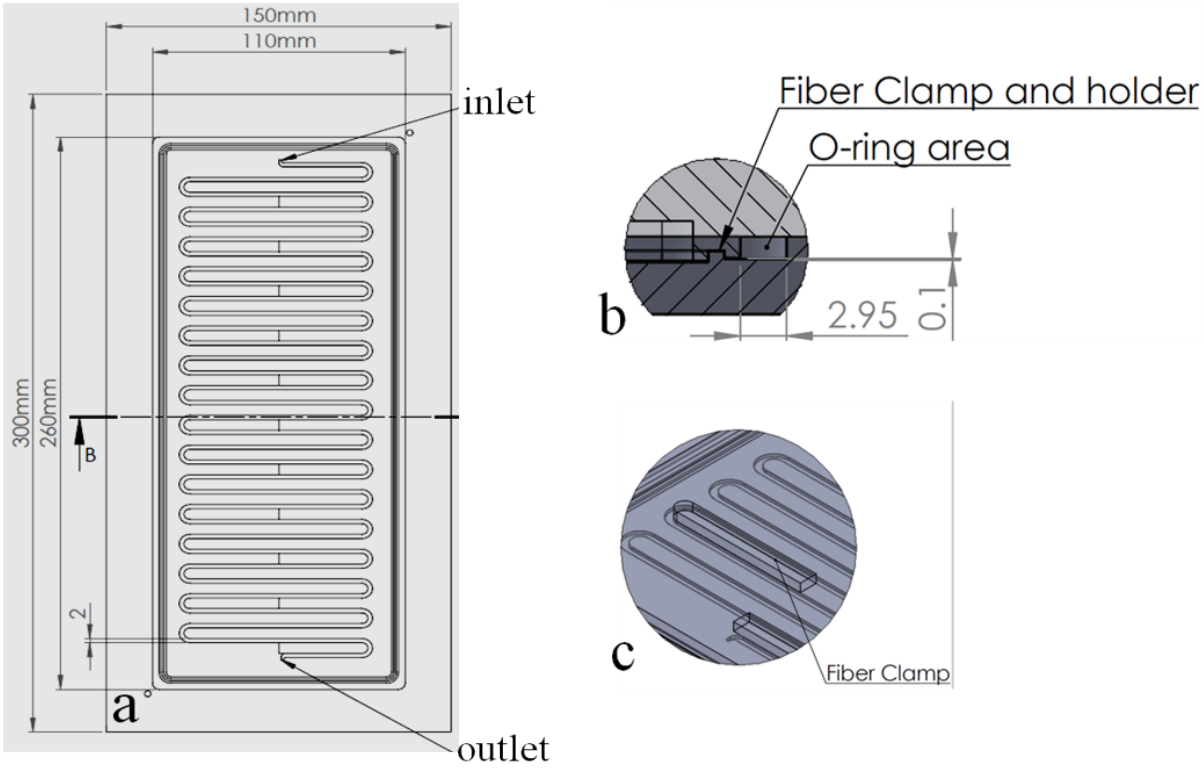

Figure S7. Schematics showing the dimensions of the fluidic systems of our artificial leaf device: (a) The bottom layer, (b) the cross-sectional view of the edge of the device and (c) a part of the top layer sitting on top of the bottom layer showing the fabric clamp to hold the composite in place.

References

1. H. T. Lokhande, N. R. Mody, K. N. Rao and M. H. Rao, *Journal of Applied Polymer Science*, 1979, **23**, 2139-2146.
2. L. Jiang, Y. Lu, X. Liu, H. Tu, J. Zhang, X. Shi, H. Deng and Y. Du, *Carbohydrate Polymers*, 2015, **121**, 428-435.