Loofah-based microalgae and cyanobacteria biocomposites for intensifying carbon dioxide capture

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Acknowledgements

This work was supported by a Petroleum Technology Development Fund, Nigeria scholarship awarded to AU and a Newcastle University Doctoral scholarship awarded to PI. Additional support was provided by National Science Foundation CBET grant 1510072. We thank Juan Nogales, Spanish National Research Council (CSIC) in Madrid, Spain for the donation of *S. elongatus* PCC 7942, Peter McParlin for assistance with microalgae culturing, Michael Foster for assistance with SEM imaging, and Jack He, Rachel Hart and Matthew Walker for laboratory assistance. We are grateful to Justin Gladman of the Shared Material Instrument Facility, Duke University for access to CT-scanning facilities.

Abstract

Microalgae and cyanobacteria have been evaluated for biological CO₂ capture from flue gases for over 40 years; however, commercial open ponds and photobioreactors suffer many drawbacks including a slow rate of CO₂ capture and high water usage. We evaluate an intensified 3D cell immobilisation approach with a small water demand, by coating latex binders onto defined surface area (947 m² m⁻³) and void space (81.78 ± 4.41%) loofah sponge scaffolds, forming porous 3D biocomposites with three microalgae species; freshwater *Chlorella vulgaris*, marine *Dunaliella salina* and *Nannochloropsis oculata*, and two strains of freshwater *Synechococcus elongatus* cyanobacteria. Binder toxicity and adhesion screening protocols were established ahead of eight weeks semi-batch and six weeks continuous CO₂ fixation trials. Acrylic and polyurethane binders were effective for microalgae, and bio-based (Replebin®) binders were suited for cyanobacteria. The highest average net CO₂ fixation rates from each species were 0.17±0.01, 0.25±0.01, 0.12±0.01, 0.68±0.18 and 0.93±0.30 g CO₂ g⁻¹_{biomass} d⁻¹ for *C. vulgaris*, *D. salina*, *N. oculata*, *S. elongatus* PCC 7942 and *S. elongatus* CCAP 1479/1A respectively. This equates to predicted CO₂ capture from scaled systems of up to 340.11±110 tCO₂ t⁻¹_{biomass} yr⁻¹. Analysis of the kinetics of CO₂ absorption and SEM imaging suggests that the cells were embedded within a polymer film that covered the scaffold. Biocomposites continuously fed with 5% CO₂ had high lipid contents approaching 70% dry weight. This biocomposite approach shows promise to intensify biological CO₂ capture and possible application in bioenergy with carbon capture and storage (BECCS).

Keywords: algae oil; biocoating; bioenergy with carbon capture and storage (BECCS); carbon capture and storage; intensified carbon capture and utilisation; latex immobilisation

1. Introduction

Carbon capture and storage (CCS) technologies are being developed as part of a suite of approaches to combat CO₂-driven climate warming [1-6] by capturing CO₂ from flue gas, particularly from major point source emitters. Post capture, the CO₂ can be injected directly into geological reservoirs for long-term sequestration. However, these processes are expensive, require suitable geological features and, paradoxically, are energy intensive with a substantial carbon footprint [7].

CCS renders the CO₂ permanently inaccessible. A parallel approach centred on carbon capture and utilisation (CCU—also known as carbon capture and conversion) aims to repurpose the captured CO₂ for use in industrial processes, e.g. ammonia and cement manufacture, or to transform it into chemically stable consumer

goods, e.g. plastics [8]. CCU approaches are advantageous inasmuch as they produce marketable goods at the end of the CO₂ value chain (potentially offsetting some of the associated CO₂ capture costs) [9]; however, this is at the expense of the degree of permanency of CO₂ sequestration [1].

It is possible to mimic nature and capture CO₂ using biological approaches by harnessing and engineering the innate CO₂-fixing capabilities of photoautotrophic organisms (plants, algae, phototrophic bacteria) [10, 11]. Phototrophs fix CO₂ into sugars using the bidirectional enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCo), with algae and cyanobacteria further benefiting from carbonic anhydrase as a carbon concentrating mechanism [12]. CO₂ captured through biological pathways is particularly amenable for CCU exploitation, potentially yielding additional benefits over and above the final carbon product, such as remediating wastewaters [13]. However, to support their carbon fixing pathways, phototrophs require exposure to light (natural or artificial) in addition to water and nutrients; thus adding significant costs to the process [10].

Numerous studies have shown that microalgae and cyanobacteria absorb CO₂ from industrial flue gas (up to 20% v/v) [14], with evidence that some species may even prefer flue gas over pure CO₂ streams [15]. These organisms (depending on species) can readily convert CO₂ into valuable metabolites e.g. lipids for biodiesel conversion or synthesis of bioplastics, with some species (especially those belonging to the *Synechococcus* and *Synechocystis* genera) capable of storing CO₂ as calcium carbonate (CaCO₃); potentially consolidating both CCU and CCS in one organism [16]. Furthermore, many microalgae and cyanobacteria can tolerate reasonably high temperatures [17], thus reducing the need for flue gas cooling prior to contact.

Approximately 70% of microalgae and cyanobacteria carbon capture trials have used open pond growth systems, which require substantial land and water, are vulnerable to biological contamination [18], and are exposed to variable weather and climate conditions, which compromise performance. Closed culture systems (photobioreactors, PBR) can overcome many of these drawbacks [19-21]; however, PBRs are not without their operational, performance, and sustainability challenges [22, 23].

If microalgae and cyanobacteria are to make meaningful contributions to CCS and CCU, new approaches that significantly intensify the rate of CO₂ absorption are needed. One such approach — the concentration, encapsulation and immobilisation of photosynthetic organisms within a thin matrix coating — draws inspiration from plant leaves and natural biofilms. These matrix-entrapped live organisms, also known as biocomposites, are being evaluated for applications such as intensifying wastewater treatment, microbial air purification, and carbon capture [13]. The increased cell loading facilitated by immobilisation can significantly enhance volumetric absorption productivity, biosorption capacity and microalgae specific activity [24]. In addition, biocomposites

require less space and are easy to handle [25, 26]. However, the mechanical limitations of the predominant immobilisation technology (hydrogels) fails to support cells for long-term storage under dry or frozen conditions, resulting in cell damage and leaching [27]. Alternative adhesive coating techniques that are robust, inexpensive and easy to implement at scale using industrial coating technology may overcome these problems. For example, Bernal [28] immobilised cyanobacteria within a latex binder wet coating on hydrated paper, yielding a 10-fold greater specific photosynthetic rate relative to suspension cultures, with CO₂ fixation sustained for up to 500 hours. Additionally, Flickinger [29] argued that latex coatings can be manipulated using bimodal blends with non-toxic nanoparticles to create sufficient porosity while still protect the immobilised cells from bacterial contamination.

In addition to paper scaffold systems, numerous synthetic scaffolds have been tested with varying degrees of success, including textiles, polyurethane foam, porous glass and ceramics [13, 30]; however, fixation rate and performance suffered from poor cell retention and/or toxicity. Natural scaffold materials may present more options. Loofah sponge (the dried fruit of the edible *Luffa* plant) offers a natural high surface area scaffold with three-dimensional (3D) geometry. Commercially, the xylem-rich fibrous structure is used as a bathing and exfoliating aide, has been studied for blending with plastics to create bioplastics packaging [31], and has been tested for numerous biotechnology applications [8, 32-35]. Microalgae and fungi have successfully been immobilised onto loofah for metal recovery from wastewater, producing enhanced biosorption capacities relative to suspension culture controls [36-39]. The highly porous structure should support excellent gas exchange and facilitate effective light transmission to a reasonable depth for immobilised phototrophs. Additionally, the moderately hydrophilic fibres [40] are effective at retaining moisture within the structure. To our knowledge, loofah-based biocomposites have yet to be assessed for gaseous carbon capture applications. Using an iterative screening process, the current work has evaluated the toxicity and effectiveness of latex binder coatings to immobilise industrially relevant live microalgae and cyanobacteria onto loofah to develop stable and inexpensive 3D biocomposites (see Fig. S1) for carbon capture applications, with an initial target operational longevity exceeding 1000 hours under both semi-batch and continuous CO₂ absorption tests.

2. Material and methods

2.1. Organisms, media and growth conditions

Three microalgae species (*Chlorella vulgaris* (CCAP 211/63) — freshwater, *Nannochloropsis oculata* (CCAP 849/1) and *Dunaliella salina* (CCAP 19/30) — marine) and two freshwater cyanobacteria strains (*Synechococcus elongatus* CCAP 1479/1A and PCC 7942) were used to produce biocomposites with petroleum (synthetic) or bio-

based binders. *C. vulgaris* and *S. elongatus* PCC 7942 were cultivated in BG11 medium, *S. elongatus* CCAP 1479/1A in JM medium, and *D. salina* and *N. oculata* in F/2 medium. All cultures were grown in 10 L polycarbonate carboys (Nalgene), with constant HEPA filtered aeration using an aquarium pump (KOI AIR 50 Blagdon), and maintained at the default conditions of our microalgae growth suite i.e. 18 ± 2 °C with a 16:8 h light:dark cycle with 2,500 lux ($\approx 30.5 \mu\text{mol m}^{-2} \text{s}^{-1}$; [41]) of illumination provided by 30W daylight-type fluorescent tubes (Sylvania Luxline Plus, n = 6).

2.2. Latex binders

Thirteen synthetic acrylic, styrene and polyurethane latex binder emulsions from a library of proprietary and commercial sources at North Carolina State University were tested (Table S1). Most of the binders have been tested in previous studies for cell immobilisations [28]. Six bio-based (plant based; Replebin®) emulsions were purchased from the AURO Paint Company, UK (Table S2) based on their distinct applications and because they are commercially available and environmentally friendly. Synthetic binders were tested with microalgae and bio-based binders were tested with cyanobacteria because some studies suggest that cyanobacteria tend to be more sensitive than microalgae to biocides present in synthetic binders [42, 43]. The pH of all binders was adjusted to within 7.5-6.5 using 0.1 and 0.5 M acetic acid. Solids content was determined using dry weight by drying at 100 °C overnight. The synthetic binders were numbered from S1 to S13 (S stands for synthetic), while the bio-based binders were numbered from B1 to B6 (B stands for bio-based). Methods for biocoatings formulation (cells plus binder), together with the approached used to assess toxicity and adhesion performance are described in supplementary material and methods.

2.3. Loofah characterisation

Loofah sponges were purchased from retail outlets. The surface area was determined as follows: a representative sample (approximately $5 \times 1 \times 1$ cm) was embedded in an epoxy resin within a silicon mold and cured for 48 h at 20 °C (100 parts EpoxiCure 2 to 23 parts hardener by weight, containing five drops of black acrylic liquid pigment to enhance contrast). Sections (1 mm thick) were cut using a Buehler IsoMet Low Speed Saw at 100 rpm. Sections were imaged using a digital HD camera (Canon EOS M10 fitted with an EF-M 15-45 mm 1:3.5-6.3 IS STM Canon zoom lens) and the loofah strand perimeter per area (mm mm^{-2}) was determined using ImageJ software.

Light penetration (transmitted photosynthetic active radiation, PAR) through different loofah sections (whole loofah in the vertical orientation, 100.8 ± 0.27 mm height; whole loofah horizontal orientation, 54.63 ± 0.72 mm diameter; half loofah horizontal orientation, 13.01 ± 0.43 mm) was measured using a Skye PAR Quantum Sensor under natural sunlight (1500 hrs BST, July 3rd, 2019, full sunlight; Fig. S2), and calculated using equation

1. The sensor was held within a lightproof tube to prevent peripheral light from distorting the readings. Ambient sunlight PAR (mean $\pm$ StDev = $1902 \pm 39 \mu\text{mol m}^{-2} \text{s}^{-1}$) was recorded before and after each loofah sample. All measurements were in triplicate.

$$\% \text{ PAR loss with depth (mm}^{-1}\text{)} = \frac{[\text{transmitted PAR/ambient PAR}]}{\text{distance}} \quad (1)$$

2.4. Semi-batch CO₂ fixation tests

To evaluate the ability of the biocomposites to absorb CO₂, semi-batch tests were carried out using samples prepared in the following way. For the microalgae species, 70 μl of biocoating formulation of known cell density (5.0% w/v cell content and 5.0% solids binder) was immobilised onto the loofah surface, whereas 600 μl of biocoating formulation (2.5% w/v cell content and 2.5% solids binder) was used for the cyanobacteria. To limit any self-shading effects, a conservative cell loading strategy was adopted relative to a comparative biocomposite study [28]. Each formulation was carefully pipetted onto one end of dried, autoclaved, pre-weighed loofah strips (dimension $1 \times 5 \text{ cm w} \times \text{h}$) and dried overnight at 20 °C. It was assumed that all cells added to the biocoating were successfully immobilised within the adhesive binder; however, the macroporous structure of the loofah (Fig. S2) caused some of the formulation to flow to waste, therefore a 100% cell loading efficiency was not achieved. To account for this, the mass of formulation dried onto the loofah was determined and normalised to a reference dried formulation. Abiotic controls comprising loofah, binder and sterile growth medium were similarly established.

For semi-batch CO₂ fixation tests (eight weeks running time), the biocomposites were placed in triplicate into 50 ml glass bottles containing 5 ml of growth medium (Fig. S3). The bottles were sealed with 20 mm butyl rubber stoppers and crimped using silver aluminium caps. Once sealed, 45 ml of 5% CO₂/air gas mixture was injected with a syringe. Triplicate suspension controls using appropriate growth media were established with a cell density equivalent to the cell loading within the biocomposite. The samples were maintained at $18 \pm 2 \text{ }^{\circ}\text{C}$ with a 16:8 light:dark photoperiod at 2500 lux. The percentage of CO₂ fixed was determined every two days by extracting the headspace with an airtight syringe and injecting into a GEOTech G100 infrared absorption CO₂ meter. The equivalent volume of CO₂ gas mixture was refreshed.

Due to cell outgrowth from the cyanobacteria in the semi-batch tests, only *C. vulgaris*, *D. salina* and *N. oculata* were evaluated for the continuous CO₂ tests (six weeks running time). Unfortunately, there were insufficient quantities of some binders following the semi-batch tests, therefore only binders S1 and S3 were tested with *C. vulgaris*, S1 and S11 with *D. salina*, and S4 and S5 with *N. oculata*.

2.5. Biocomposite microstructure analysis

Raw loofah was imaged in 3D using a Nikon XTH 225 ST high resolution x-ray computed tomography scanner with a 225 kV UltraFocus Reflection Based Signal and Tungsten Target with a Perkin Elmer 1620 AN3 CS CT detector. The biocomposites were analysed using scanning electron microscopy (SEM) before and after adhesion testing and after the eight weeks semi-batch net CO₂ fixation test. Samples were dried at 105 °C for 3 h and stored in a desiccator before being attached to 12 mm diameter pin stubs using carbon tape. The instrument was a Hitachi TM 3000 SEM equipped with a backscattered electrons system for digital image acquisition. All biocomposites were observed in two or more randomised locations using a 5 or 15 kV accelerating voltage. Each location was imaged multiple times using sequential magnifications ranging from 15× to 30,000× to characterise surface topography, cell distribution and available pore space.

2.6. Analysis of CO₂ transfer kinetics

The kinetics of CO₂ transport from the gas phase to the cells immobilised with coatings on loofah scaffolds were analysed using the CO₂ absorption rate data from the semi-batch tests and by solving the unsteady state diffusion equation. The rate of CO₂ (R_{CO_2}) is given by equation 2, where Ka_c is the volumetric transfer coefficient (s⁻¹) and c is the concentration in the gas or liquid phase (mols m⁻³).

$$R_{CO_2}(\text{mols m}^3 \text{ s}^{-1}) = Ka_c c \quad (2)$$

Equation 2 is written for CO₂ transport from the bulk liquid or gas phase to inside the cell assuming that the CO₂ concentration in the cell is zero due to photosynthesis. For the suspension controls, the cells were surrounded by liquid and the volumetric transfer coefficient was calculated by solving equation 3, where c^L is the CO₂ concentration in the liquid (mols m⁻³), $D_{CO_2}^L$ is the diffusivity of CO₂ in the culture medium (1.92 x 10⁻⁹ m² s⁻¹), t is the time (s) and z is the vertical distance in the vial measured from the gas-liquid interface (m).

$$\frac{\partial c^L}{\partial t} = D_{CO_2}^L \frac{\partial^2 c^L}{\partial z^2} - Ka_c c^L \quad (3)$$

Equilibrium was assumed to exist at the interface between the gas and the culture medium. The equilibrium was modelled using Henry's Law. For the biocomposite samples the cells are surrounded by gas and the volumetric transfer coefficient was calculated by solving equation 4, where c^G is the CO₂ concentration in the gas (mols m⁻³), $D_{CO_2}^G$ is the diffusivity of CO₂ in the gas phase (1.6 x 10⁻⁵ m² s⁻¹) and x and y are horizontal distances perpendicular to the vertical axis of the sample vial (m).

$$\frac{\partial c^G}{\partial t} = D_{CO_2}^G \left(\frac{\partial^2 c^G}{\partial x^2} + \frac{\partial^2 c^G}{\partial y^2} \right) - K a_c c^G \quad (4)$$

Equations 3 and 4 were solved over the 48 hour period between fresh gas injections ($c_0^G = 2.04 \text{ mols m}^{-3}$). The value of the transfer coefficient was adjusted until the concentrations predicted by equations 3 and 4 matched the values measured in the gas at the end of the 48 hours period. Data from day 14 of the semi-batch test onwards were used. Full details about the solution of equations 3 and 4 are given in the supplementary materials (Figs S4-S7, Table S3).

2.7. Continuous CO₂ fixation tests

After completing and analysing the data from the semi-batch tests, an experiment was set up to demonstrate that biocomposites can be used to continuously capture CO₂ and convert it into a useable product. The lipid producing species *C. vulgaris*, *D. salina* and *N. oculata* were chosen for these tests since we sought to demonstrate that the biocomposites could produce a commercially viable product. Approximately 1 g of wet cell paste (see supplementary material and methods) of known cell density ($1.13 \times 10^9 \text{ cells/ml}$) was mixed by vortex with 1 ml of pH adjusted synthetic latex binder and 50 ml of growth medium. Each formulation was poured into individual 100 ml beakers containing loofah ($50 \times 55 \text{ mm}$, $h \times d$) and dried at 30 °C in a shaking incubator (250 rpm for 48 to 60 h) (Fig. S8). After drying, the biocomposites were placed into duplicate 100 ml Duran glass bottles connected to a continuous CO₂ flow rig (Fig. S9) constructed from a polypropylene tube (length = 91.5 cm, diameter = 63.0 mm, wall thickness = 5.0 mm, internal diameter = 53.0 mm). Each bottle was connected via 6.35 mm (¼ inch) PTFE tubes to a continuous flow of 5% CO₂/air mixture at $40 \text{ cm}^3 \text{ min}^{-1}$. Gas flow was regulated using a Cole-Parmer MC-series gas mass flow controller and distributed equally to each bottle using needle valves. The gas outlet was collected using a 1L airtight sampling gas bag and analysed every 24 h using a G100 GeoTech CO₂ meter. Approximately 5 ml of growth medium were refreshed every 72 h. Growth conditions matched the semi-batch CO₂ fixation test. Abiotic controls were prepared and tested in the same manner.

The opacity of the binders, coupled with the structural complexity of the loofah, made direct cell counts impractical, therefore growth was estimated based on mass changes. The net quantity of CO₂ fixed by the immobilised cells (dry biomass basis) was calculated based on the total immobilised biomass (after normalising to the abiotic and growth medium controls). Biomass was determined based on the difference in weight of the loofah before and after immobilisation after binder density was taken into account. As CO₂ readings were taken every two days, the net CO₂ fixation rate was calculated using equation 5, where P is gas pressure (kPa), V is gas volume (l), R is the ideal gas constant (J K mol^{-1}) and T is temperature (K).

$$\text{net CO}_2 \text{ fixation rate (mol CO}_2 \text{ g}_{\text{biomass}}^{-1} \text{ day}^{-1}) = \frac{\% \text{ CO}_2 \text{ fixed} \times \frac{PV}{RT}}{\text{Immobilised dried biomass (g)} \times 2 \text{ days}} \quad (5)$$

To calculate the CO₂ fixation rate per unit surface area, the following assumptions were made: the percentage of void area was the same throughout the structure, i.e. the perimeter to area ratio is equivalent to surface area per volume (mm⁻² mm⁻³); loofah does not consume CO₂; the biocoating formulation was uniformly distributed across the loofah surface; and there was no outgrowth of cells beyond the estimated surface area.

2.8. Lipid extraction

At the end of the continuous CO₂ test the total lipid content of the *C. vulgaris* and *D. salina* biocomposites were analysed using a modified Bligh and Dyer (1959) gravimetric method [44] (*N. oculata* was not included due to a substantial loss of cells during the experiment). Before lipid extraction, the biocomposites were dried at 105 °C for 24 h to remove any absorbed growth medium, then freeze dried using liquid nitrogen and ground by pestle and mortar. For the first extraction, approximately 40 ml methanol: chloroform: dH₂O (2:1:0.8 v/v/v) was added to 50 ml polypropylene centrifuge tubes with the samples and left to react for 48 h. The samples were filtered to separate solid residue and supernatant and transferred into a new 50 ml tube. For the second extraction, 30 ml of the solvent mix was added and left to react for 24 h. The topmost liquid layer (methanol and water) in each extraction was carefully removed and the chloroform/lipid layer was transferred to a pre-weighed vial. Once completely dried, the vial was weighed to calculate the percentage lipid per biomass dry weight. Total lipids were also extracted from the abiotic and loofah-only controls and subtracted from the measured lipid content of biocomposites.

2.9. Statistics

Data were statistically analysed using Minitab 17 and all methods were chosen based on Smith [45]. Toxicity and adhesion data were not normally distributed and were analysed using Kruskal-Wallis tests with Mann-Whitney U-tests. Cumulative net CO₂ fixation data from the semi-batch test were analysed by one-way analysis of variance (ANOVA) with Tukey's Honest Significant Difference post-hoc test at 34 and 46 days for microalgae and cyanobacteria respectively (corresponding to the final data obtained from the suspension controls) and at the termination of the experiment (56 days). Cumulative net CO₂ fixation data from the continuous test were analysed by ANOVA with Tukey's post-hoc test for all the biocomposites at the final time period of the suspension controls (34 days) and at the termination of the experiment (42 days). The percentage lipid content data were also analysed using ANOVA.

3. Results

3.1. Loofah characterisation

The loofah had a surface area available for coating equivalent to $947 \text{ m}^2 \text{ m}^{-3}$ with a void space (for gas exchange and light penetration) of $81.78 \pm 4.41\%$. The loss in absolute transmitted PAR was greatest in the whole loofah in the horizontal orientation (from 1927 ± 20 to $71 \pm 22 \mu\text{mol m}^{-2} \text{ s}^{-1}$), followed by the whole loofah in the vertical orientation (from 1851 ± 11 to $98 \pm 2 \mu\text{mol m}^{-2} \text{ s}^{-1}$), and the half loofah (from 1930 ± 2 to $146 \pm 3 \mu\text{mol m}^{-2} \text{ s}^{-1}$); however, when converted to the percentage loss in PAR per unit distance the half loofah was the worst performer followed by the horizontal whole loofah then the vertical whole loofah (Table S4).

3.2. Latex binder toxicity and adhesion screening

A decision matrix based on toxicity and adhesion performance was applied to select the most suitable binders for subsequent net CO_2 fixation tests (Fig. 1) [46]. Most of the synthetic binders clustered at the extremes of the matrix, whereas the bio-based binders were more evenly distributed. This was due to the majority of synthetic latexes significantly inhibiting microalgae growth relative to the suspension control (Fig. S10a-c) while bio-based binders B5 and B6 significantly enhanced the growth of *S. elongatus* CCAP 1479/1A and PCC 7942 by roughly 1.5- and 3-fold respectively (Fig. S10d-e). During the seven day toxicity test, toxic binders showed photo-bleaching unlike non-toxic binders (as an example, see Fig. S11). Without complete knowledge of proprietary commercial binder surface chemistry and formulations it is not possible to determine the relative contribution of each component to the observed toxicity.

For adhesion tests, thirteen acrylic and polyurethane latex binders were tested with *C. vulgaris*, however a limited supply of the synthetic binders necessitated that only the five least toxic binders were tested with *N. oculata* and *D. salina*. The binders retaining most cells were S9, S1 and S11 for *C. vulgaris*, *N. oculata* and *D. salina* respectively, with each releasing less than 1% of cells (Fig. S12). Binders B1 and B5 were the leading bio-based binders with 0.61% of *S. elongatus* PCC 7942 and 3.49% of CCAP 1479/1A released (Fig. S12).

SEM analysis of the biocomposite structure was conducted before and after cell immobilisation. In addition to covering the loofah strands, the binders tended to form between the pore spaces potentially reducing the overall porosity of the structure with possible implications for gas exchange and light penetration. Fig. 2 shows contrasting examples of effective (displaying a smooth surface; Fig. 2b) and poorly performing synthetic binders (cracked during drying; Fig. 2c). Cracked binders could not adequately retain the cells within the structure as indicated by poor adhesion data. The equivalent *S. elongatus* biocomposites are presented in Fig. 3 and Fig. S13. The least effective binders (B4 for CCAP 1479/1A; Fig. 3a-d and B3 for PCC 7942; Fig. S13a-d) deteriorated

rapidly, whereas binders B5 (CCAP 1479/1A) (Fig. 3e-h) and B1 (PCC 7942) (Fig. S13e-h) retained the cells on the loofah surface in the same form as prior to the adhesion test.

As a result from the screening, three synthetic binders were selected per microalga; binder S1 was selected for all three species, S8 for *D. salina* and *N. oculata*, S3 and S10 for *C. vulgaris*, S11 for *D. salina* and S4 for *N. oculata*. Binder B6 was selected for *S. elongatus* PCC 7942 and binder B5 for CCAP 1479/1A.

3.3. CO₂ fixation rates in the semi-batch tests

With excellent pH and temperature tolerances [47] loofah may be a useful candidate scaffold for capture from gases containing CO₂, for example the flue gas from combustion processes. A liquid absorption capacity of up to 18.4 g g⁻¹ of fibers [36] should deliver sufficient hydration and sustenance to bound cells via capillary action over extended periods, in a mechanism similar to the cellulosic sheet biofilm-based photobioreactor described by Hamano and co-workers [48]. To test this, we utilised semi-batch and continuous CO₂ fixation tests over extended time periods (eight and six weeks respectively). Fig. 4 shows the cumulative net CO₂ fixation of the biocomposites over the 8-week semi-batch testing period. The microalgae and cyanobacteria suspension controls were terminated after 34 and 46 days respectively due to culture failure — likely due to a combination of nutrient exhaustion and self-shading, due in part to the initial high inoculum density required to match the biocomposite cell loading. In contrast, only a very limited number of loofah treatments failed to establish, specifically one *C. vulgaris* replicate with both binders S3 and S10. In all cases, net CO₂ fixation rates from the biocomposites substantially exceeded the suspension controls (Fig. 4, Table 1). For *C. vulgaris* (Fig. 4a), binder S1 had the highest net fixation rate (2.52 ± 0.10 mmol g⁻¹ d⁻¹ — where g refers to g of phototroph biomass not total biocomposite) followed by S3 (1.73 ± 1.50 mmol g⁻¹ d⁻¹) and S10 (1.16 ± 1.05 mmol g⁻¹ d⁻¹). A reduction in net fixation rate was noted after approximately 33 days, however each biocomposite remained functional up to the termination of the test. There were no significant differences between binders at 34 days or at the termination of the experiment — likely an artefact of reduced statistical power following the loss of one replicate for two of the binders. For *D. salina* (Fig. 4b, Table 1), binder S1 (1.96 ± 0.48 mmol g⁻¹ d⁻¹) was significantly outperformed by both binders S11 (2.99 ± 0.41 mmol g⁻¹ d⁻¹) and S8 (2.95 ± 0.25 mmol g⁻¹ d⁻¹) at both time points. There was no evidence of a reduction in performance by the end of the trial. There were no statistical differences between the performances of the *N. oculata* biocomposites at either time point (Fig. 4c, Table 1) with the highest rate (2.71 ± 0.24 mmol g⁻¹ d⁻¹) achieved with binder S4. As with the *D. salina* trials, there was no evidence of any reduction in performance over the eight weeks. *S. elongatus* CCAP 1479/1A yielded the highest net fixation rate of all the biocomposites (21.18 ± 6.87 mmol g⁻¹ d⁻¹), over ten times higher than its suspension control (2.08 ± 0.13 mmol g⁻¹ d⁻¹). The *S. elongatus*

PCC 7942 biocomposite was $15.38 \pm 4.06 \text{ mmol g}^{-1} \text{ d}^{-1}$, almost 9-fold higher than its suspension control ($1.77 \pm 0.12 \text{ mmol g}^{-1} \text{ d}^{-1}$). There were no significant differences between the biocomposite performances at either time point (Fig. 4d, Table 1).

3.4 CO₂ transfer kinetics

Using the experimental data from the semi-batch tests, the volumetric CO₂ transfer coefficients for the suspended culture controls and the biocomposites were calculated. These data are presented in Table 2 and Figure 5 and show that the CO₂ transfer coefficient is lower for the biocomposites compared to the suspended cultures. This was expected as by incorporating the cells into the binder an additional transfer resistance has been added. The transfer coefficients are higher for biocomposites incorporating the cyanobacteria compared to microalgae because the cells are smaller and a greater number were included per unit volume of coating, leading to a higher cell surface area for CO₂ transfer.

In an attempt to determine the nature of the mass transfer resistance in the biocoatings, the data in Table 2 were analysed in more depth. First, using cell surface areas for the suspended cultures, values of the coefficient for transfer through the cell walls and cell internals were calculated, k_{cell} ($\text{mols m}^{-2} \text{ s}^{-1} \cdot (\text{mols m}^{-3})^{-1}$). Next, using cell surface areas for the biocomposites, values of the coefficient for transfer through the binder layer, cell walls and cell internals were calculated, k_{BC} . It was assumed that the resistances due to the cell and the binder were additive as shown in equation 6.

$$\frac{1}{k_{\text{BC}}} = \frac{1}{k_{\text{binder}}} + \frac{1}{k_{\text{cell}}} \quad (6)$$

Equation 6 was used to calculate values of the transfer coefficient for the binder, k_{binder} . The thickness of the binder layer applied to the loofah was calculated based on the assumption that the volume of the biocoating formulation in the semi-batch tests (600 μl) was uniformly distributed over the surface of the binder. This gave an average thickness for the binder layer of 26 μm . Being a solid, the resistance of the binder layer is given by equation 7. In this equation, δ_{B} , is the thickness of the binder layer and D_{B} is the CO₂ diffusivity in the binder layer ($\text{m}^2 \text{ s}^{-1}$).

$$k_{\text{binder}} = \frac{D_{\text{B}}}{\delta_{\text{B}}} \quad (7)$$

Equations 6 and 7 were used to calculate values for the diffusivity in the binder. The results of these calculations (Table 3) show that the CO₂ diffusivities in the binder are of the order of $10^{-15} - 10^{-13} \text{ m}^2 \text{ s}^{-1}$. By comparing these values with the diffusivity of gases in microporous solids ($\sim 10^{-8} \text{ m}^2 \text{ s}^{-1}$) [49], diffusivities of gases in liquids ($\sim 10^{-9} \text{ m}^2 \text{ s}^{-1}$) and the diffusivity of CO₂ in polymer films ($\sim 10^{-13} \text{ m}^2 \text{ s}^{-1}$) [50], they imply that the cells are covered by a solid layer of polymer, a conclusion which is supported by the SEM images in Figure 3.

3.5 Continuous CO₂ fixation tests and lipid content

In the continuous CO₂ exposure tests, as with the semi-batch test, each biocomposite substantially outperformed its respective suspension control (*C. vulgaris*: ANOVA, $F=25.47$, $DF=2$, $P=0.013$; *D. salina*: ANOVA, $F=41.39$, $DF=2$, $P=0.007$; *N. oculata*: ANOVA, $F=9.60$, $DF=2$, $P=0.05$) when analysed at the final time period of the suspension controls (34 days). However, there were no significant differences between biocomposites of the same species at the termination of the experiment (42 days). The highest cumulative CO₂ fixation was from *D. salina* with binder S11 (239.15 ± 11.29 mmol g⁻¹) (Fig. 6b), corresponding to a daily cumulative CO₂ fixation at six weeks of 5.69 ± 0.24 mmol g⁻¹ d⁻¹ (Table 4), which was approaching 18 times higher than the suspension culture (0.32 ± 0.05 mmol g⁻¹ d⁻¹). The highest CO₂ fixation from *C. vulgaris* was in combination with binder S3 (165.25 ± 6.53 mmol g⁻¹ or 3.93 ± 0.16 mmol g⁻¹ d⁻¹) followed by S1 (150.37 ± 29.76 mmol g⁻¹) (Fig. 6a, Table 4). *N. oculata* fixed 162.43 ± 54.80 mmol g⁻¹ with binder S4 (Fig. 6c). However, there was a poor adhesive relationship between *N. oculata* and the binders that allowed cells to escape the structure and grow in the pool of liquid growth medium; therefore, the *N. oculata* CO₂ fixation data should be interpreted cautiously. Comparing between the semi-batch and continuous tests (discounting *N. oculata*), the continuous approach yielded consistently greater CO₂ fixation rates, equating to relative performance improvements of 42% for *C. vulgaris* with binder S1, 90% for *D. salina* with binder S11, 117% for *C. vulgaris* with binder S1 and 127% for *C. vulgaris* with binder S3.

Immobilisation significantly increased the lipid content of both *C. vulgaris* (ANOVA, $F=33.28$, $DF=2$, $P=0.009$) and *D. salina* (ANOVA, $F=68.14$, $DF=2$, $P=0.003$) compared to their suspension controls. The *C. vulgaris* biocomposites had dry weight lipid contents of $69.38 \pm 1.27\%$ and $51.96 \pm 11.84\%$ for binders S1 and S3 respectively, compared with $14.53 \pm 0.47\%$ for the suspension control (Fig. 7a). The values for *D. salina* were $36.1 \pm 4.72\%$ and $66.22 \pm 6.02\%$ for binders S1 and S11 respectively, compared with $14.86 \pm 0.20\%$ for the suspension controls (Fig. 7b). These were at the upper end of reported lipid contents for both species [51-53]. Photosynthetic conversion efficiencies were calculated for the six weeks trial, ranging between 0.019 ± 0.002 and $0.036 \pm 0.001\%$ (See Table S4). These efficiencies are low (see [54] who report 8-10% efficiencies) due to the low light levels, modest temperature and non-renewal of nutrients.

4. Discussion

Loofah was selected as a candidate 3D scaffold for a low water demand photosynthetic biocomposite for carbon capture applications. Light transmission was not uniform through the loofah, with differences in the percentage loss in PAR affected by the void volumetric space at the centre of the whole loofah allowing greater light penetration per unit depth (Fig. S2). The half loofah therefore had a greater overall strand density (strands mm⁻³)

relative to the whole loofah in either orientation. It should be noted that these values are based on direct sunlight, whereas the CO₂ fixation tests were conducted under artificial light at a considerably lower light intensity (30.5 μmol m⁻² s⁻¹). This approximates to light levels of 19.7 μmol m⁻² s⁻¹ penetrating to the centre of the loofah in the semi-batch test and 22.7 μmol m⁻² s⁻¹ for the continuous CO₂ fixation test assuming no additional light loss from the biocoating.

The CO₂ absorption rates across the biocomposites were significantly higher than their suspension controls due to a higher CO₂ concentration gradient and loofah surface area compared to suspended cultures, even in treatments that experienced a failed replicate. That aside, biocomposite performance was stable across treatments as evidenced by the consistent and tight standard deviations. The biocoatings were applied manually, with between replicate variability largely explained by the extent and lack of uniform coverage of the loofah strands, with some pooling of the biocoating on the less illuminated side of the loofah strand—this could partly be ameliorated using spray application. Typically, CO₂ is taken up for photosynthesis by algae and cyanobacteria as bicarbonate (HCO₃⁻), although there is evidence of direct CO₂ uptake depending on their environmental parameters, taxonomic features, concentration of carbon sources and pH of culture medium [55, 56]. The sorbed water via the thin film of coating in the biocomposites should support a steep CO₂ concentration gradient and rapid solubility of CO₂, allowing efficient uptake by the cells. In contrast, the suspension controls lacked dynamic mixing and relied only on the slow driving force of diffusion which limits mass transfer efficiency as the molecules are required to pass through bulk liquid water before reaching the cells [57]. A similar scenario will exist for O₂ outgassing (to prevent photorespiration), with the biocomposites presenting a more efficient system [55, 58, 59]. Additionally, as nutrients were not renewed during the tests, increased CO₂ uptake rates could also stem from nutrient limitation [60], as the immobilised cells were reliant upon capillary action via loofah to access the growth medium whereas the suspended cells were permanently bathed in nutrients. This scenario would similarly explain the large differences in lipid content between treatments as nutrient limitation (particularly nitrogen), is a renowned initiator of triglycerides synthesis in microalgae [61].

Based on the semi-batch test data, the biocomposites can theoretically capture CO₂ at rates of between 40.47 ± 1.61 to 340.11 ± 110.32 tCO₂ t⁻¹_{biomass} yr⁻¹ (Table 1). The continuous CO₂ test yielded substantive performance gains (ranging from 42 to 127%), and potentially yielding upwards of 600 kg of lipids; however, we lack comparative data for the cyanobacteria (the best performing biocomposites under semi-batch testing conditions). If we assume similar relative performance gains, then *S. elongatus* under continuous exposure could potentially scale to between 350.7 and 560.6 tCO₂ t⁻¹_{biomass} yr⁻¹ for PCC 7942 and 483 and 776.6 tCO₂ t⁻¹_{biomass} yr⁻¹

¹ for CCAP 1479/1A, although the lower lipid biosynthesis capability of *S. elongatus* would limit lipid yields to approximately 110 kg [62]. Thus, other end products made up of carbohydrates or protein should be targeted instead of lipid for *S. elongatus* biocomposites.

It is challenging to compare the current study with other microalgae-based carbon capture technologies, due to a lack of standardised conditions between experiments, coupled with variation in the reported metrics (Table 5). The majority of studies have been conducted with suspension cultures at various scales, with few using a biocomposite or biofilm approach. Our data compare favourably with studies that report CO₂ fixation as g CO₂ g⁻¹_{biomass} d⁻¹. However, the fixation rates achieved with *S. elongatus* biocomposites were 20 times lower than Bernal et al.'s paper-based acrylate copolymer latex coating biocomposites with immobilised *S. elongatus* PCC 7002 [28]. This is likely due to cell loading differences; 2.5% versus 50% v/v, i.e. a 20-fold difference. Vaz et al. [63] used the initial dry weight biomass of 0.23 g to cultivate *Chlorella fusca* on polymeric nanofibers whereas we used 0.05 g of *C. vulgaris* for the continuous CO₂ absorption test. The net CO₂ fixation rate achieved from their study was 0.27 g CO₂ g⁻¹ d⁻¹ which is broadly comparable to our *C. vulgaris* + binder S3 data (0.17 ± 0.01 g CO₂ g⁻¹ d⁻¹). It should be noted that we assume that the binder application is perfectly efficient, i.e. that all cells in the biocoating are immobilised to the loofah. In reality, some cells are lost to waste by our current coating technique forming non-uniform coating thickness in the biocomposite structure. Therefore, the CO₂ fixation rates per g biomass will be underestimated. Improvements may therefore be made by increasing cell loading (i.e. using a denser biocoating); however, Flickinger et al. [64] mentioned that a threshold cell loading for latex binders (as yet undefined, and likely species and binder specific) should not be exceeded as it may compromise porosity and adhesion. Further, substantially increased cell loading may compromise performance through excessive competition for water, light, nutrients and CO₂. A recent study suggested that it is possible to increase the porosity of the latex to produce stable biocoating using non-toxic non-film forming nanoparticles [65]; offering a potential route to improve the performance of our biocomposite systems.

There were few significant cell releases from the biocomposites during the semi-batch CO₂ testing with synthetic latex binders (Fig. S14). *C. vulgaris* and *N. oculata* remained within the binders (Fig. S14c&i), although the *D. salina* biocomposite (Fig. S14f) did display some pitting from cell loss (possibly during the drying process). However, most cells of both cyanobacteria strains grew out of the bio-latex binders after four weeks into the growth medium (see Fig. S15). During the continuous CO₂ test it was *N. oculata* rather than *D. salina* that experienced adhesion and retention issues. The loss of cells to the liquid growth medium in both tests would have

reduced the overall CO₂ fixation if the released cells reverted to a photosynthetic rate equivalent to the suspension controls.

Conditions inside the sample vials during the tests may have compromised binder integrity as the biocomposites were continuously exposed to moisture that may have altered their adhesive properties [66]. For the cyanobacteria, the bio-latex appeared to enhance the growth rate as previously observed in the toxicity test (Fig. S10d-e), with the growing biomass leading to partial binder failure and release of the cells. This observation poses something of a paradox for mass microalgae/cyanobacteria production systems. Ordinarily, open ponds and PBRs are designed to maximise biomass production by delivering optimised growth conditions; however, rapid biomass production may be suboptimal for immobilised systems. For biocomposites, an unusual ‘maintenance of viability’ approach is warranted whereby the nutrient system must deliver conditions supportive of active metabolism but sufficiently suboptimal to slow or even eliminate cell division [64], such as has been achieved for *Escherichia coli* [67], *Rhodospseudomonas palustris* [68] and *Gluconobacter oxydans* [69]. When combining cells with binders, it appeared to initiate a cell morphology change for *S. elongatus*, transiting from a rod-shaped to a coccoid form (Fig. S16). The implications of this for immobilised cultivation are unknown; however, similar observations have been made for *S. elongatus* and other bacteria with suggestions that the behaviour may regulate photosynthesis and cell division under stress [40, 70, 71].

The findings from the semi-batch tests appear to show that the cells are covered with a thin polymer layer which protects the cells. The cell retention capabilities of many of the latex binders should stimulate future biocomposite coating technology exploration, including expanding the use of microalgae ‘milking’ to recover biochemicals (including lipids) without permanently compromising cell health [72], and specifically as a platform for the adoption and safe environmental deployment of metabolically engineered microorganisms for industrial and environmental applications [73, 74]. On a similar note, the species assayed in this study are evolved for a planktonic existence (i.e. living in suspension in the water column). The immobilisation process compels the cells to live in a film, i.e. to become benthic — a living state for which they may be genetically ill-equipped. As indicated, the cyanobacteria responded by changing their cell morphology. Options exist to metabolically engineer the organisms whereby the transition from planktonic to benthic lifestyles may be routine as has previously been done for *S. elongatus* [75-77], or they may be engineered for improved adhesive capabilities as reported for *E. coli* [78, 79]. Equally, synthetic biology approaches could be used to address resource limitation effects, such as combating self-shading [80]. On the theme of entraining planktonic cells to a forced benthic existence, future work may also wish to explore natural biofilm forming species as candidates for biocomposite manufacture, as

many such species naturally synthesise extracellular polymeric substances (EPS) that support adhesion and biofilm integrity [81, 82]. However, to sound a note of caution, many such species also utilise EPS as a biolubricant to aid gliding motility (c.f. many raphid diatoms) which could act to breakdown the biocomposite. In such instances the need for a robust binder may be even greater, although we have previously observed that a raphid pennate diatom (*Navicula pelliculosa*; In-na and Caldwell, unpublished) readily escapes binders by exploiting EPS to reduce adhesion. Additional complications may arise with natural biofilm formers, as dispersion is an intrinsic part of a biofilm cycle, particularly in response to nutrient stress [83, 84]. Such a scenario could lead to epidemic biocomposite failure. EPS could also provide a ready substrate for bacterial growth which may compromise the health and longevity of the biocomposite. Whilst the use of planktonic species may seem counterintuitive, there are compelling arguments why they may have fewer operational problems than natural biofilm forming taxa.

In this study, we developed microalgae and cyanobacteria based biocomposites for carbon capture over sustained durations, utilising loofah sponge as a cheap and sustainable scaffold. Cell immobilisation greatly enhanced net CO₂ fixation relative to suspension controls, with most biocomposites showing little or no reduction in performance. The cyanobacteria biocomposites significantly outperformed their microalgae comparators; however, this may be due to either the greater surface area of cells in cyanobacteria biocomposites or the differing binders used between the groups (petroleum-based synthetic binders for microalgae versus bio-based binders for the cyanobacteria). The microalgae also had a high lipid content compared to their suspension controls, potentially opening loofah based biocomposites for applications in bioenergy with carbon capture and storage (BECCS). These 3D coated biocomposites overcome some of the disadvantages of open pond and photobioreactor growth systems, particularly in terms of water consumption. Further optimisation is required to increase CO₂ absorption, for example by increasing cell loading, reducing the mass transfer resistance of the polymer layer over the cells and conducting tests under high PAR intensity sunlight rather than low intensity artificial light. With further improvements, including metabolic engineered systems, it may not be unreasonable to predict that loofah based biocomposites (particularly with cyanobacteria) could capture in the region of 1000 tCO₂ t⁻¹_{biomass} yr⁻¹.

Author contributions

The study was conceived by all authors. Data were collected by AU, PI and AW and analysed by AU, PI, AW, GC and JL. The manuscript was written by AU, PI, GC and JL, and edited and approved by AW and MF.

Compliance with ethical standards

Funding: This study was funded by the National Science Foundation CBET (grant no. 1510072) and the Petroleum Technology Development Fund, Nigeria.

Conflict of Interest: Abbas A. Umar declares that he has no conflict of interest. Pichaya In-na declares that she has no conflict of interest. Adam D. Wallace declares that he has no conflict of interest. Michael C. Flickinger declares that he has no conflict of interest. Gary S. Caldwell declares that he has no conflict of interest. Jonathan G.M. Lee declares that he has no conflict of interest.

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

Figure legends

Fig. 1 Decision matrix derived from toxicity and adhesion data for (a) *C. vulgaris*, (b) *D. salina*, (c) *N. oculata*, (d) *S. elongatus* PCC 7942 and (e) CCAP 1479/1A. Refer to Tables S1 and S2 for binder details.

Fig. 2 SEM images of a representative loofah scaffold (a) before and (b) after cell immobilisation with effective and (c) ineffective synthetic binders. Magnification: 40 $\times$.

Fig. 3 SEM images of *S. elongatus* CCAP 1479/1A biocomposites before and after adhesion testing. Red boxes indicate where the cells were immobilised with binders. Binder B4 exhibiting poor cell retention (before = a&c, after = b&d), binder B5 exhibiting good cell retention (before = e&g, after = f&h). Magnification: 4000 $\times$ (a, b, e, f) and 9000 $\times$ (c, d, g, h).

Fig. 4 Cumulative net CO₂ fixation (**mean $\pm$ StDev**) of (a) *C. vulgaris*, (b) *D. salina*, (c) *N. oculata* and (d) *S. elongatus* biocomposites compared with their suspension culture controls from the eight weeks semi-batch CO₂ fixation test.

Fig. 5 Comparison of the volumetric CO₂ transfer coefficients for suspended and biocomposite cultures of cyanobacteria and microalgae.

Fig. 6 Cumulative net CO₂ fixation (**mean $\pm$ StDev**) of (a) *C. vulgaris*, (b) *D. salina*, and (c) *N. oculata* biocomposites compared with their suspension culture controls from the six weeks continuous CO₂ fixation test.

Fig. 7 Percentage biomass lipid content (dry weight) (**mean $\pm$ StDev**) of (a) *C. vulgaris* and (b) *D. salina* biocomposites compared with their suspension culture controls at the end of the six weeks continuous CO₂ fixation test.

Table 1 Net semi-batch CO₂ fixation rates (mean ± StDev) for the best performing biocomposite for each species compared with their suspension culture controls: *C. vulgaris* + S1; *D. salina* + S11; *N. oculata* + S4; *S. elongatus* PCC 7942 + B6; CCAP 1479/1A + B5. Data are presented in a range of formats to better enable comparison with other published studies. A theoretical scaled CO₂ capture (as tonne CO₂ captured per tonne of biomass per year) from an up-scaled version of the biocomposite system is also presented.

Phototroph	Net CO ₂ fixation rates					Theoretical scaled CO ₂ capture (tCO ₂ t ⁻¹ _{biomass} yr ⁻¹)
	Suspension (mmol CO ₂ g ⁻¹ _{biomass} d ⁻¹)	Biocomposite (mmol CO ₂ g ⁻¹ _{biomass} d ⁻¹)	Biocomposite (mmol CO ₂ m ⁻² d ⁻¹)	Biocomposite (g CO ₂ m ⁻² d ⁻¹)	Biocomposite (g CO ₂ g ⁻¹ _{biomass} d ⁻¹)	
<i>C. vulgaris</i>	0.92 ± 0.03	2.52 ± 0.10	4.66 ± 0.18	0.20 ± 0.01	0.11 ± 0.01	40.47 ± 1.61
<i>D. salina</i>	0.40 ± 0.06	2.99 ± 0.41	5.53 ± 0.76	0.24 ± 0.03	0.13 ± 0.02	48.01 ± 6.58
<i>N. oculata</i>	0.48 ± 0.05	2.71 ± 0.24	5.01 ± 0.44	0.22 ± 0.02	0.12 ± 0.01	43.52 ± 3.85
<i>S. elongatus</i> PCC 7942	1.77 ± 0.03	15.38 ± 4.06	7.72 ± 2.04	0.34 ± 0.09	0.68 ± 0.18	246.97 ± 65.20
<i>S. elongatus</i> CCAP 1479/1A	2.08 ± 0.13	21.18 ± 6.87	6.15 ± 2.00	0.27 ± 0.09	0.93 ± 0.30	340.11 ± 110.32

Table 2 Comparison of the volumetric CO₂ transfer coefficients for suspended culture and the biocomposites.

Phototroph	Cell surface area (m ² x 10 ¹¹)	N _{cell} x 10 ⁻⁷	Suspended culture		Biocomposite	
			a _c (m ² m ⁻³)	K a _c (s ⁻¹ x 10 ⁶)	a _c (m ² m ⁻³)	K a _c (s ⁻¹ x 10 ⁶)
<i>S. elongatus</i> PCC 7942 ^a	2.15	3160	128060	14.6 ± 0.7	135744	5.4 ± 0.5
<i>S. elongatus</i> CCAP 1479/1A ^b	1.26	4490	106458	62.7 ± 1.1	112846	5.9 ± 0.6
<i>C. vulgaris</i>	7.85 - 31.4	0.59 - 5.14	920 - 2016	6.3 ± 5.3	921 - 2019	2.4 ± 0.5
<i>D. salina</i>	80.4 - 181	0.044 - 0.16	397 - 635	2.7 ± 2.5	397 - 637	1.6 ± 0.1
<i>N. oculata</i>	1.26 - 5.03	9.50 - 81.1	2383 - 5088	3.7 ± 2.9	2386 - 5098	2.0 ± 0.2

a – Calculated from rod-shaped cells with an assumption that all cells had a diameter and length of 2 and 4 µm.

b – Calculated from coccoid cells with an assumption that all the cells had a diameter of 2 µm.

Table 3 Values of the binder film diffusivity calculated from experimental transfer coefficients.

Phototroph	k_{cell} ($\text{m s}^{-1} \times 10^{10}$)	k_{BC} ($\text{m s}^{-1} \times 10^{10}$)	K_{binder} ($\text{m s}^{-1} \times 10^{10}$)	D_{B} ($\text{m}^2 \text{s}^{-1} \times 10^{15}$)
<i>S. elongatus</i> PCC 7942	1.1	0.39	0.60	1.6
<i>S. elongatus</i> CCAP 1479/1A	5.9	0.52	0.57	1.5
<i>C. vulgaris</i>	50	19	31	80
<i>D. salina</i>	55	32	75	200
<i>N. oculata</i>	11	6.2	14	35

Table 4 Net continuous CO₂ fixation rates (mean ± StDev) for each biocomposite for *C. vulgaris* and *D. salina* compared with their suspension culture controls. A theoretical scaled CO₂ capture (as tonne CO₂ captured per tonne of biomass per year) from an up-scaled version of the biocomposite system is also presented.

Biocomposite	Net CO ₂ fixation rates					Theoretical scaled CO ₂ capture (tCO ₂ t ⁻¹ biomass yr ⁻¹)
	Suspension (mmol CO ₂ g ⁻¹ biomass d ⁻¹)	Biocomposite (mmol CO ₂ g ⁻¹ biomass d ⁻¹)	Biocomposite (mmol CO ₂ m ⁻² d ⁻¹)	Biocomposite (g CO ₂ m ⁻² d ⁻¹)	Biocomposite (g CO ₂ g ⁻¹ biomass d ⁻¹)	
<i>C. vulgaris</i> + S3	0.65 ± 0.02	3.93 ± 0.16	2.38 ± 0.10	0.10 ± 0.01	0.17 ± 0.01	63.11 ± 2.57
<i>C. vulgaris</i> + S1		3.58 ± 0.50	2.17 ± 0.30	0.10 ± 0.01	0.16 ± 0.02	57.49 ± 8.03
<i>D. salina</i> + S11	0.32 ± 0.05	5.69 ± 0.24	3.44 ± 0.15	0.15 ± 0.01	0.25 ± 0.01	91.37 ± 3.85
<i>D. salina</i> + S1		4.31 ± 0.67	2.61 ± 0.40	0.11 ± 0.02	0.19 ± 0.03	69.21 ± 41.91

Table 5 Comparison of the highest CO₂ fixation rates for semi-batch and continuous tests relative to selected literature values, including the model species *Arabidopsis thaliana* as a reference plant. We made approximate calculations and conversions based on the information available if data were not explicitly stated within a paper in an appropriate form.

Species and strain	Type	System description	CO ₂ fixation rate		Ref
			(mmol CO ₂ m ⁻² d ⁻¹)	(g CO ₂ g ⁻¹ biomass d ⁻¹)	
<i>Arabidopsis thaliana</i> (wild type)	Angiosperm	Open gas exchange chamber (ambient CO ₂) at 25 °C; light: 300 μmol m ⁻² s ⁻¹	432	0.25	[85]
<i>Monoraphidium dybowskii</i> LB50	Freshwater chlorophyte	200 m ² open raceway at ambient temperature and CO ₂	750	0.37	[40]
<i>Synechococcus</i> PCC7002	Cyanophyte	Paper-based biocomposites with 20% v/v CO ₂ at 25 °C; light: 100 μmol m ⁻² s ⁻¹	136	0.22	[28]
<i>Oedogonium crispum</i>	Freshwater chlorophyte (macroalgae)	15,000 L culture tank at ambient temperature, CO ₂ enriched regulated by culture pH; light: 230 μmol m ⁻² s ⁻¹	85	0.45	[86]
<i>Chlorella fusca</i> LEB 111	Freshwater chlorophyte	Cultivated on CO ₂ adsorbent nanofibers in 0.45 L PBR at 25 °C; light: 41.6 μmol m ⁻² s ⁻¹	-	0.27	[63]
<i>C. vulgaris</i>	Freshwater chlorophyte	15 L tubular reactor at 25 °C with continuous 8% v/v CO ₂ (100 l h ⁻¹); light: >twice daylight	-	0.91	[87]
<i>Nannochloropsis gaditana</i>	Marine ochrophyte	1.5 L tubular reactor at 25 °C with continuous 8% v/v CO ₂ (100 l h ⁻¹); light: >twice daylight	-	1.61	[87]
<i>S. elongatus</i> CCAP 1479/1A	Cyanophyte	Immobilised with binder B5 on loofah at 18 °C; light: 16:8 photoperiod at 30.5 μmol m ⁻² s ⁻¹ with 5% v/v CO ₂ in semi-batch at day 56	6.15	0.93	This study
<i>Dunaliella salina</i>	Marine chlorophyte	Immobilised with binder S11 on loofah at 18 °C; light: 16:8 photoperiod at 30.5 μmol m ⁻² s ⁻¹ with continuous 5% v/v CO ₂ at day 42	3.44	0.25	This study

Fig. 1

Loofah strand

Binder

Cracked binder

Fig 2

Fig 3

Fig 4

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Fig 5

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Fig 6

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Fig 7

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Loofah-based microalgae and cyanobacteria biocomposites for intensifying carbon dioxide capture

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Supplementary materials and methods

Contact toxicity test

After pH adjustment, binders were formulated to have 5% w/w solids content using deionised water (dH₂O). Approximately 1 ml of each binder was mixed with 1 ml of cell culture in 24-multiwell plates. The cell/binder mixtures were cultured for seven days (20 °C, 16:8 light:dark photoperiod at 2,500 lux), after which the samples were diluted by a factor of 20 using dH₂O and cell counts were conducted using an improved Neubauer Hawksley haemocytometer relative to suspension controls.

Cell adhesion test

A wet cell paste (WCP) was obtained for each test species by centrifuging 2 L of culture of known cell density. The centrifugation conditions varied depending on species; 5 mins at 966 RCF (Relative Centrifugal Force) for *C. vulgaris*, 10 mins at 1717 RCF for *N. oculata*, 2 mins at 1717 RCF for *D. salina*, and 30 mins at 1717 RCF for the cyanobacteria. Loofah were autoclaved, then dried at 105 °C for 3 h. After drying, they were stored overnight in a desiccator prior to immobilisation. For immobilisation, the loofah was cut into approximately 1 × 1 × 1 cm cubes and placed in 24-multiwell plates. The binder mixtures were formulated as per the contact toxicity test. Each binder was mixed with 2.5% and 5.0% v/v of WCP and vortexed for 10 to 20 seconds (Vortex Genie 2, Scientific Industries, Inc.) to form biocoatings. During mixing of the marine microalgae WCP, the artificial seawater reacted with the latex binders, subsequently the artificial seawater was premixed with the binders before mixing with the marine WCP. Approximately 2 ml of biocoating was pipetted onto each loofah cube and dried at 30 °C for 24 h. Once formed, each biocomposite was submerged in 2 ml of growth medium and shaken in darkness at 80 rpm for 1, 24, 48 and 72 h. The growth medium was refreshed at each stated time interval and any cells released from the loofah surface were counted by haemocytometer and converted to a percentage of the cells loaded onto the loofah scaffold. A weighted decision matrix technique was used to identify the most suitable binders for each species based on toxicity and adhesion affinity to the loofah scaffold. Toxicity was given a higher weighting (ratio 3:2) as the maintenance of cell viability was deemed the more important characteristic.

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51 **Fig. S1** Theoretical biofilm arrangement for loofah based biocomposites comprising cells and binder particles
 52 on a loofah scaffold.

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A void space at the
centre of the loofah

Fig. S2 Illustrating the porosity and orientation of loofah for the light penetration test. CT scans of loofah (a-c): (a) loofah external view in longitudinal orientation, (b) internal view in longitudinal orientation, (c) internal view in transverse section. (d-g) Orientation for light tests. The red line indicates the light path relative to the sun. The PAR sensor was encased in a lightproof tube to prevent peripheral light affecting the readings; (d) whole loofah in vertical orientation, (e) whole loofah in horizontal orientation, (f) half loofah (bracket denotes the loofah thickness), and (g) an example of void spaces within the loofah centre.

Table S1 Name, coding and available compositional details of the synthetic petroleum based latex binders. N/A = not available.

Binder name	Binder code	Main component	% Solid	Additional component	Additional component %
Rhoplex SF-3122	S1	Acrylic polymer	60-61	Aqua ammonia	0.2 Max
JP 912	S2	Acrylic polymer	51.1	Individual residual monomers	<0.1
Rhoplex SG -10M	S3	Butyl acrylate/Methyl methacrylate	51.3	N/A	N/A
PD-0413	S4	N/A	80.4	N/A	N/A
PN-3415-W	S5	N/A	46.55	N/A	N/A
Genflo 8045	S6	Modified styrene butadiene	57.1	N/A	N/A
Rhoplex SG-10	S7	Butyl/methyl methacrylate	49-51	Aqua ammonia	<=0.2
HB 3691 M	S8	Polyester polyurethane	48.4	N/A	N/A
Baycusan C-1000	S9	Polyester polyurethane	40	N/A	N/A
Baycusan C-1004	S10	Polyester polyurethane	40	N/A	N/A
Baymedix CH-120 polyurethane resin dispersion	S11	Polyurethane polymer	34.7	N/A	N/A
Nuplex Setaqua 6776	S12	Acrylic polymer	42.8	N/A	N/A
Rhoplex SF-012	S13	Acrylic polymer	43-44	Aqua ammonia	<=0.1

78 **Table S2** Name, coding and available compositional details of the bio-based latex binders.

Binder name	Binder code	Main components	% Solid
AURO Clear lacquer glossy	B1	Water, colophony glycerol ester with organic acids, mineral fillers, sunflower oil, linseed oil, drying agents (cobalt-free), castor stand oil, surfactants, amino soap, silicic acid, cellulose, fatty acids	29.10
AURO Clear lacquer matt silk	B2	Water, colophony glycerol ester with organic acids, mineral fillers, sunflower oil, linseed oil, drying agents (cobalt-free), castor stand oil, surfactants, amino soap, silicic acid, cellulose, fatty acids	19.52
AURO Woodstain transparent	B3	Water, linseed oil, colophonium glycerine ester with organic acids, mineral fillers, surfactants, mineral pigments, silicic acids, dryers (cobalt-free), castor stand oil, sunflower seed oil, titanium dioxide, ammonium soaps, cellulose, fatty acids	13.68
AURO Color wash binder	B4	Water, ammonium soap, ammonium carbonate, alcohol	9.01
AURO Ecological dispersion wall paint	B5	Mineral fillers, water, Replebin, titanium dioxide, cellulose, surfactants made of rapeseed-, castor oil, ammonia and thiazoles	52.85
AURO Ecological dispersion wall paint	B6	Mineral fillers, water, Replebin, titanium dioxide, cellulose, surfactants made of rapeseed-, castor oil, ammonia and thiazoles	52.57

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Fig S3 A schematic sequence of the process used to immobilise microalgae or cyanobacteria on loofah sponge using latex binders for the semi-batch CO₂ fixation test.

Simulation of control vial and biocomposite experiments to estimate mass transfer resistances.

1.0 Control vial model

In this section the simulation of diffusion in the control vials, filled with gas and a suspended microalgae or cyanobacteria culture, is described. The purpose of this work was to establish a rate constant for the removal of CO₂ by the cells suspended in culture medium. The removal of CO₂ by the cells is assumed to be a first order process.

$$\text{rate of CO}_2 \text{ removal by cells} = K a_c c_{\text{CO}_2}$$

This assumes that either mass dominates or that the reaction consuming CO₂ is first order. The diffusion model in conjunction with experimental data were used to determine values for the volumetric transfer coefficient K a_c. A second diffusion model was used to simulate vials with biocomposite and the values of the volumetric transfer coefficient were compared.

1.1 Differential mass balances

In the gas phase we assume diffusion is the only mass transfer mechanism. A mass balance and the application of Fick's first law leads to equation 1.1.

$$\frac{\partial c^G}{\partial t} = D_{\text{CO}_2}^G \frac{\partial^2 c^G}{\partial z^2} \quad (1.1)$$

Assuming that:

1. there is no mixing in the liquid phase and that diffusion is the only mass transfer mechanism. Diffusion flux is in the axial direction; and
2. CO₂ removal by the cells is represented by a reaction that is first order with respect to the concentration of CO₂ in the liquid phase.

Using these assumptions a mass balance in the liquid phase leads to equation 1.2.

$$\frac{\partial c^L}{\partial t} = D_{\text{CO}_2}^L \frac{\partial^2 c^L}{\partial z^2} - K a_c c^L \quad (1.2)$$

To solve these equations we have to spatially discretize them and solve the resulting set of ordinary differential equations. The vial is split into slices as shown in Fig. S4.

Since diffusion is the only transport mechanism for the CO₂, the second order differentials in equations 1.1 and 1.2 can be represented using second order central differences. Equations 1.1 and 1.2 are discretized to give equation 1.3 and 1.4.

$$\frac{\partial c_i^G}{\partial t} = D_{\text{CO}_2}^G \left(\frac{c_{i+1}^G - 2c_i^G + c_{i-1}^G}{\Delta z^2} \right) \quad (1.3)$$

$$\frac{\partial c_j^L}{\partial t} = D_{\text{CO}_2}^L \left(\frac{c_{j+1}^L - 2c_j^L + c_{j-1}^L}{\Delta z^2} \right) - K a_c c_j^L \quad (1.4)$$

Fig. S4 The vial compartmentalised into a numerical grid.

1.2 Boundary conditions

There are three boundaries within the vial; the top of the vial, the gas-liquid interface, and the base of the vial.

1.2.1 Top and bottom of the vial

At the top and bottom of the vial, the wall prevents diffusion so the diffusion flux is zero. We used a forward difference at the top of the vial and a backwards difference at the bottom of the vial to represent the concentration gradient as shown in equations 1.5 and 1.6.

$$J_0^G = -D_{CO_2}^G \frac{\partial c^G}{\partial z} \Big|_0 = 0 = -D_{CO_2}^G \left(\frac{-3c_0^G + 4c_1^G - c_2^G}{2\Delta z} \right) \quad (1.5)$$

$$J_M^L = -D_{CO_2}^L \frac{\partial c^L}{\partial z} \Big|_M = 0 = -D_{CO_2}^L \left(\frac{c_{M-2}^L - 4c_{M-1}^L + 3c_M^L}{2\Delta z} \right) \quad (1.6)$$

Equations 1.5 and 1.6 were solved for the gas concentration at the top of the vial (c_0^G) and the liquid concentration at the base of the vial (c_M^L), as given by equations 1.7 and 1.8.

$$c_0^G = \frac{4}{3}c_1^G - \frac{1}{3}c_2^G \quad (1.7)$$

$$c_M^L = \frac{4}{3}c_{M-1}^L - \frac{1}{3}c_{M-2}^L \quad (1.8)$$

1.2.2 Interface between gas and liquid

At the interface between the gas and the liquid (surface S in Fig. S4) we assume that equilibrium exists between the phases. In this case the equilibrium is given by Dalton's law and Henry's law, as given by equation 1.7.

$$y_S P = c_S^G RT = H c_S^L \quad (1.7)$$

Mass balances for the cell above the interface in the gas phase (eqn. 1.8) and the cell below the interface in the liquid phase (eqn. 1.9) are written in terms of the flux of CO₂ instead of the concentration gradient.

$$\frac{\partial c_N^G}{\partial t} = \frac{J_N^G - J_i}{\Delta z_G} \quad (1.8)$$

$$\frac{\partial c_1^L}{\partial t} = \frac{J_i - J_1^L}{\Delta z_L} \quad (1.9)$$

The diffusion fluxes in the gas (J_N^G) and liquid (J_1^L) phases are estimated using Fick's law with second order differences, as given by equations 1.10 and 1.11.

$$J_N^G = -D_{CO_2}^G \frac{\partial c^G}{\partial z} \Big|_N = -D_{CO_2}^G \left(\frac{c_{N-2}^G - 4c_{N-1}^G + 3c_N^G}{2\Delta z_G} \right) \quad (1.10)$$

$$J_1^L = -D_{CO_2}^L \frac{\partial c^L}{\partial z} \Big|_1 = -D_{CO_2}^L \left(\frac{-3c_1^L + 4c_2^L - c_3^L}{2\Delta z_L} \right) \quad (1.11)$$

The flux crossing the interface is calculated by applying two film theory around the interface so that the concentrations at point N in the gas phase and point 1 in the liquid phase can be used instead of interfacial concentrations. The flux is given by equation 1.12.

$$J_i = K_{ov} \left(\frac{c_N^G RT}{H} - c_1^L \right) \quad (1.12)$$

The overall mass transfer coefficient, K_{ov} , is given by equation 1.13.

$$\frac{1}{K_{ov}} = \left(\frac{\Delta z_L}{D_{CO_2}^L} + \frac{\Delta z_G}{D_{CO_2}^G} \frac{RT}{H} \right) \quad (1.13)$$

Substituting from equations 1.10, 1.11 and 1.12 into equations 1.8 and 1.9 leads to equations 1.14 and 1.15 which describe the way the CO₂ concentration in the cells next to the interface change with time.

$$\frac{\partial c_N^G}{\partial t} = \frac{1}{\Delta z_G} \left[D_{CO_2}^G \left(\frac{-c_{N-2}^G + 4c_{N-1}^G - 3c_N^G}{2\Delta z_G} \right) - K_{ov} \left(\frac{c_N^G RT}{H} - c_1^L \right) \right] \quad (1.14)$$

$$\frac{\partial c_1^L}{\partial t} = \frac{1}{\Delta z_L} \left[K_{ov} \left(\frac{c_N^G RT}{H} - c_1^L \right) - D_{CO_2}^L \left(\frac{3c_1^L - 4c_2^L + c_3^L}{2\Delta z_L} \right) \right] - a_c k c_1^L \quad (1.15)$$

1.3 Making the equations dimensionless

Defining the following characteristic variables

$$\bar{c} = \frac{c}{c_0} \quad \bar{t} = \frac{t}{\tau} \quad \text{where} \quad \tau = \frac{\Delta z_0 z_0}{D_0} \quad \bar{z} = \frac{z}{z_0} \quad \Delta z_0 = \frac{z_0}{N+1+M}$$

The mass balances in the gas and liquid phases are transformed using dimensionless variables. The mass balances are given for the first cell in the gas layer, the cells in the middle of the gas layer, cell N in the gas layer, cell 1 in the liquid layer and cell M-1 in the liquid layer.

Point 1 in the gas layer

$$\frac{\partial \bar{c}_1^G}{\partial \bar{t}} = \frac{2}{3} \frac{\Delta z_0}{z_0} \frac{D_{CO_2}^G}{D_0} \left(\frac{\bar{c}_2^G - \bar{c}_1^G}{\Delta \bar{z}_G^2} \right) \quad (1.16)$$

Points 2 to N-1 in the gas layer

$$\frac{\partial \bar{c}_i^G}{\partial t} = \frac{\Delta z_0}{z_0} \frac{D_{CO_2}^G}{D_0} \left(\frac{\bar{c}_{i+1}^G - 2\bar{c}_i^G + \bar{c}_{i-1}^G}{\Delta z_G^2} \right) \quad (1.17)$$

Point N in the gas layer

$$\frac{\partial \bar{c}_N^G}{\partial t} = \frac{\Delta z_0}{z_0} \frac{1}{\Delta z_G} \left[\left(\frac{D_{CO_2}^G}{D_0} \right) \left(\frac{-\bar{c}_{N-2}^G + 4\bar{c}_{N-1}^G - 3\bar{c}_N^G}{2\Delta z_G} \right) - \left(\frac{z_0}{D_0} \right) K_{ov} \left(\frac{\bar{c}_N^G RT}{H} - \bar{c}_1^L \right) \right] \quad (1.18)$$

Point 1 in the liquid layer

$$\frac{\partial \bar{c}_1^L}{\partial t} = \frac{\Delta z_0}{z_0} \frac{1}{\Delta z_L} \left[\left(\frac{z_0}{D_0} \right) K_{ov} \left(\frac{\bar{c}_N^G RT}{H} - \bar{c}_1^L \right) - \left(\frac{D_{CO_2}^L}{D_0} \right) \left(\frac{3\bar{c}_1^L - 4\bar{c}_2^L + \bar{c}_3^L}{2\Delta z_L} \right) \right] - \left(\frac{z_0 \Delta z_0}{D_0} \right) K a_C \bar{c}_1^L \quad (1.19)$$

Points 2 to M-2 in the liquid layer

$$\frac{\partial \bar{c}_j^L}{\partial t} = \frac{\Delta z_0}{z_0} \frac{D_{CO_2}^L}{D_0} \left(\frac{\bar{c}_{j+1}^L - 2\bar{c}_j^L + \bar{c}_{j-1}^L}{\Delta z_L^2} \right) - \left(\frac{z_0 \Delta z_0}{D_0} \right) K a_C \bar{c}_j^L \quad (1.20)$$

Point M-1 in the liquid layer

$$\frac{\partial \bar{c}_{M-1}^L}{\partial t} = \frac{2}{3} \frac{\Delta z_0}{z_0} \frac{D_{CO_2}^L}{D_0} \left(\frac{\bar{c}_{M-2}^L - \bar{c}_{M-1}^L}{\Delta z_L^2} \right) - \left(\frac{z_0 \Delta z_0}{D_0} \right) K a_C \bar{c}_{M-1}^L \quad (1.21)$$

1.4 Solving the equations

Equations were set up and solved by numerical integration using Mathematica version 12. The following protocol was adopted to ensure that the models represented the experimental situation.

Saturating the liquid with CO₂

Run 1, saturate the liquid with CO₂ at atmospheric concentrations of CO₂. Runs 2-4, put initial gas concentration up 5 mol%. Final gas and liquid compositions were the same between runs 3 and 4.

Implementing the light dark cycles.

A piecewise function was used to simulate the light-dark cycles of the growth room. It was assumed that the gas in the vial was changed at 15:00 and that the lights were switched off at 21:00 for eight hours. The schedule is given in Table S3.

Table S3 Lighting schedule during the trial.

Day	Time	Hours	Lights on/off
1	15:00	0	on
1	21:00	6	off
2	05:00	14	on
2	21:00	30	off
3	05:00	38	on

These are based on assumptions: 1) one dimensional diffusion in the vial, 2) all vials contain the same number of cells, and 3) all biocomposite samples contain the same number of cells as the suspended cultures in control vials

2.0 Diffusion model for vials containing biocomposite

2.1 Mass balance equations and discretization

In the gas surrounding the biocomposite sample a mass balance on an element with dimensions $\Delta x \Delta y \Delta z$ ($\Delta z = 1$) leads to equation 2.1.

$$\frac{\partial c^G}{\partial t} = -\frac{\partial J_x}{\partial x} - \frac{\partial J_y}{\partial y} \quad (2.1)$$

In the gas within the biocomposite sample, a mass balance on an element with dimensions $\Delta x \Delta y \Delta z$ ($\Delta z = 1$), including diffusion and CO_2 consumption due to photosynthesis leads to equation 2.2.

$$\frac{\partial c^G}{\partial t} = -\frac{\partial J_x}{\partial x} - \frac{\partial J_y}{\partial y} - a_{BC} k c^G \quad (2.2)$$

Using Fick's first law of diffusion to replace the diffusion fluxes in equations 2.1 and 2.2 leads to equations 2.3 and 2.4.

$$\frac{\partial c^G}{\partial t} = D_{CO_2}^G \left(\frac{\partial^2 c^G}{\partial x^2} + \frac{\partial^2 c^G}{\partial y^2} \right) \quad (2.3)$$

$$\frac{\partial c^G}{\partial t} = D_{CO_2}^G \left(\frac{\partial^2 c^G}{\partial x^2} + \frac{\partial^2 c^G}{\partial y^2} \right) - a_{BC} k c^G \quad (2.4)$$

Equations 2.3 and 2.4 are solved by discretizing the spatial derivatives on the numerical grid shown in Fig. S5. Due to rotational symmetry only a quarter of the vial and biocomposite sample have to be simulated. In Fig. S5 the black lines represent the biocomposite sample, within these lines equation 2.4 is used for simulation. Outside the black lines equation 2.3 is used. The yellow lines represent symmetry boundaries and the green lines the wall of the vial. Using second order central second differences to replace the concentration gradients in equations 2.3 and 2.4 leads to equations 2.5 and 2.6.

$$\frac{\partial c_{i,j}^G}{\partial t} = D_{CO_2}^G \left(\frac{c_{i+1,j}^G - 2c_{i,j}^G + c_{i-1,j}^G}{\Delta x^2} + \frac{c_{i,j+1}^G - 2c_{i,j}^G + c_{i,j-1}^G}{\Delta y^2} \right) \quad (2.5)$$

$$\frac{\partial c_{i,j}^G}{\partial t} = D_{CO_2}^G \left(\frac{c_{i+1,j}^G - 2c_{i,j}^G + c_{i-1,j}^G}{\Delta x^2} + \frac{c_{i,j+1}^G - 2c_{i,j}^G + c_{i,j-1}^G}{\Delta y^2} \right) - a_{BC} k c_{i,j}^G \quad (2.6)$$

Fig. S5 The numerical grid.

Equation 2.3 or 2.4 is written for every point on the numerical grid within the green boundary. This leads to a set of ordinary differential equations that are solved over time to follow the evolution of CO₂ concentration.

2.2 Boundary conditions

There are two boundary conditions that are applied at the boundaries of the numerical grid.

At the symmetry boundaries: $\frac{\partial c^G}{\partial n} = 0$

At the vial wall: $J_x = J_y = 0$

The remainder of this section will deal with the application of these boundary conditions to the numerical grid shown in Fig. S5.

Symmetry boundaries

Along the x-axis symmetry boundary, diffusion in the y-direction is normal to the boundary, and on the y-axis symmetry boundary diffusion in the x-direction is normal to the boundary. Therefore we can write that:

$$\left. \frac{\partial c^G}{\partial y} \right|_{y=0} = 0 \quad \Rightarrow \quad \left. \frac{\partial^2 c^G}{\partial y^2} \right|_{y=0} = 0$$

$$\left. \frac{\partial c^G}{\partial x} \right|_{x=0} = 0 \quad \Rightarrow \quad \left. \frac{\partial^2 c^G}{\partial x^2} \right|_{x=0} = 0$$

This simplifies the mass balance equations as follows. At the x-axis symmetry boundary equations 2.7 and 2.8 are used, depending on whether the point is inside or outside the biocomposite sample.

$$\frac{\partial c_{i,j}^G}{\partial t} = D_{CO_2}^G \left(\frac{c_{i+1,j}^G - 2c_{i,j}^G + c_{i-1,j}^G}{\Delta x^2} \right) \quad (2.7)$$

$$\frac{\partial c_{i,j}^G}{\partial t} = D_{CO_2}^G \left(\frac{c_{i+1,j}^G - 2c_{i,j}^G + c_{i-1,j}^G}{\Delta x^2} \right) - a_{BC} k c_{i,j}^G \quad (2.8)$$

At the y-axis symmetry boundary, equations 2.9 and 2.10 are used, depending on whether the point is inside or outside the biocomposite sample.

$$\frac{\partial c_{i,j}^G}{\partial t} = D_{CO_2}^G \left(\frac{c_{i,j+1}^G - 2c_{i,j}^G + c_{i,j-1}^G}{\Delta y^2} \right) \quad (2.9)$$

$$\frac{\partial c_{i,j}^G}{\partial t} = D_{CO_2}^G \left(\frac{c_{i,j+1}^G - 2c_{i,j}^G + c_{i,j-1}^G}{\Delta y^2} \right) - a_{BC} k c_{i,j}^G \quad (2.10)$$

At the centre of the vial and the biocomposite sample there are two symmetry boundaries. At this single point there are no concentration gradients in the x or y direction and the mass balances simplifies to give equation (2.11).

$$\frac{\partial c_{i,j}^G}{\partial t} = -a_{BC} k c_{i,j}^G \quad (2.11)$$

Fig. S6 Vial wall boundaries.

Vial boundaries

The different types of vial wall boundary cells in the numerical grid are shown in Fig. S6. These are horizontal wall Fig. S6(a), vertical wall Fig. S6(b) and a corner Fig. S6(c).

For the horizontal wall the flux normal to the wall is in the y-direction. The boundary condition at the wall is

$$J_{i,j+1} = -D_{CO_2}^L \frac{\partial c^G}{\partial y} \Big|_{i,j+1} = 0 = -D_{CO_2}^G \left(\frac{c_{i,j-1}^G - 4c_{i,j}^G + 3c_{i,j+1}^G}{2\Delta z} \right) \quad (2.12)$$

246 solving equation 2.12 for $c_{i,j+1}^G$ gives $c_{i,j+1}^G = \frac{4}{3}c_{i,j}^G - \frac{1}{3}c_{i,j-1}^G$. Substituting this into equation 2.3 leads to equation
 247 2.13.

$$248 \quad \frac{\partial c_{i,j}^G}{\partial t} = D_{CO_2}^G \left(\frac{c_{i+1,j}^G - 2c_{i,j}^G + c_{i-1,j}^G}{\Delta x^2} + \frac{2}{3} \left[\frac{c_{i,j-1}^G - c_{i,j}^G}{\Delta y^2} \right] \right) \quad (2.13)$$

249 For the vertical wall the flux normal to the wall is in the x-direction. The boundary condition at the wall is

$$250 \quad J_{i+1,j} = -D_{CO_2}^L \frac{\partial c^G}{\partial y} \Big|_{i+1,j} = 0 = -D_{CO_2}^G \left(\frac{c_{i-1,j}^G - 4c_{i,j}^G + 3c_{i+1,j}^G}{2\Delta z} \right) \quad (2.14)$$

251 solving equation 2.14 for $c_{i+1,j}^G$ gives $c_{i+1,j}^G = \frac{4}{3}c_{i,j}^G - \frac{1}{3}c_{i-1,j}^G$. Substituting this into equation 2.3 leads to equation
 252 2.15.

$$253 \quad \frac{\partial c_{i,j}^G}{\partial t} = D_{CO_2}^G \left(\frac{2}{3} \left[\frac{c_{i-1,j}^G - c_{i,j}^G}{\Delta x^2} \right] + \frac{c_{i,j+1}^G - 2c_{i,j}^G + c_{i,j-1}^G}{\Delta y^2} \right) \quad (2.15)$$

254 For a corner, the flux in the y-direction at point (i,j+1) and the flux in the x-direction at point (i+1,j) are both zero.

255 Solving equations 2.12 and 2.14 and substituting the results into equation 2.3 leads to equation 2.16.

$$256 \quad \frac{\partial c_{i,j}^G}{\partial t} = \frac{2D_{CO_2}^G}{3} \left(\frac{c_{i-1,j}^G - c_{i,j}^G}{\Delta x^2} + \frac{c_{i,j-1}^G - c_{i,j}^G}{\Delta y^2} \right) \quad (2.16)$$

257 There are a further two types of wall point, those which lie on the symmetry boundaries. If a wall point lies on the
 258 x-axis symmetry boundary then equation 2.17 is used and if it lies on the y-axis then equation 2.18 is used.

$$259 \quad \frac{\partial c_{i,j}^G}{\partial t} = \frac{2}{3} D_{CO_2}^G \left(\frac{c_{i-1,j}^G - c_{i,j}^G}{\Delta x^2} \right) \quad (2.17)$$

$$260 \quad \frac{\partial c_{i,j}^G}{\partial t} = \frac{2}{3} D_{CO_2}^G \left(\frac{c_{i,j-1}^G - c_{i,j}^G}{\Delta y^2} \right) \quad (2.18)$$

261 2.3 Making the equations dimensionless

262 Defining the following characteristic variables

$$263 \quad \bar{c} = \frac{c}{c_0} \quad \bar{t} = \frac{t}{\tau} \quad \bar{x} = \frac{x}{r_0} \quad \bar{y} = \frac{y}{r_0}$$

264 where

$$265 \quad \tau = \frac{\Delta r_0 r_0}{D_0} \quad \Delta r_0 = \min [\Delta x, \Delta y]$$

266 According to whether biocomposite is present and applying the boundary conditions, equations 2.3, 2.4, 2.5, 2.6,
 267 2.7, 2.8, 2.9, 2.11, 2.13, 2.14, 2.15 and 2.16 are the mass balances that will be made dimensionless using these
 268 characteristic variables. The dimensionless mass balances (equations 2.19-28) were solved to give the
 269 concentration in the vial as a function of time.

270 Gas only – type “G”

$$271 \quad \frac{\partial \bar{c}_{i,j}^G}{\partial \bar{t}} = \frac{D_{CO_2}^G \Delta r_0}{D_0 r_0} \left(\frac{\bar{c}_{i+1,j}^G - 2\bar{c}_{i,j}^G + \bar{c}_{i-1,j}^G}{\Delta \bar{x}^2} + \frac{\bar{c}_{i,j+1}^G - 2\bar{c}_{i,j}^G + \bar{c}_{i,j-1}^G}{\Delta \bar{y}^2} \right) \quad (2.19)$$

272 Biocomposite present – type “B”

$$273 \quad \frac{\partial \bar{c}_{i,j}^G}{\partial \bar{t}} = \frac{D_{CO_2}^G \Delta r_0}{D_0 r_0} \left(\frac{\bar{c}_{i+1,j}^G - 2\bar{c}_{i,j}^G + \bar{c}_{i-1,j}^G}{\Delta x^2} + \frac{\bar{c}_{i,j+1}^G - 2\bar{c}_{i,j}^G + \bar{c}_{i,j-1}^G}{\Delta y^2} \right) - \left(\frac{\Delta r_0 r_0}{D_0} \right) a_{BC} k \bar{c}_{i,j}^G \quad (2.20)$$

274 Symmetry boundary (x-axis) gas only – type “GSX”

$$275 \quad \frac{\partial \bar{c}_{i,j}^G}{\partial \bar{t}} = \frac{D_{CO_2}^G \Delta r_0}{D_0 r_0} \left(\frac{\bar{c}_{i+1,j}^G - 2\bar{c}_{i,j}^G + \bar{c}_{i-1,j}^G}{\Delta x^2} \right) \quad (2.21)$$

276 Symmetry boundary (x-axis) biocomposite present – type “BSX”

$$277 \quad \frac{\partial \bar{c}_{i,j}^G}{\partial \bar{t}} = \frac{D_{CO_2}^G \Delta r_0}{D_0 r_0} \left(\frac{\bar{c}_{i+1,j}^G - 2\bar{c}_{i,j}^G + \bar{c}_{i-1,j}^G}{\Delta x^2} \right) - \left(\frac{\Delta r_0 r_0}{D_0} \right) a_{BC} k \bar{c}_{i,j}^G \quad (2.22)$$

278 Symmetry boundary (y-axis) gas only – type “GSY”

$$279 \quad \frac{\partial \bar{c}_{i,j}^G}{\partial \bar{t}} = \frac{D_{CO_2}^G \Delta r_0}{D_0 r_0} \left(\frac{\bar{c}_{i,j+1}^G - 2\bar{c}_{i,j}^G + \bar{c}_{i,j-1}^G}{\Delta y^2} \right) \quad (2.23)$$

280 Symmetry boundary (y-axis) biocomposite present – type “BSY”

$$281 \quad \frac{\partial \bar{c}_{i,j}^G}{\partial \bar{t}} = \frac{D_{CO_2}^G \Delta r_0}{D_0 r_0} \left(\frac{\bar{c}_{i,j+1}^G - 2\bar{c}_{i,j}^G + \bar{c}_{i,j-1}^G}{\Delta y^2} \right) - \left(\frac{\Delta r_0 r_0}{D_0} \right) a_{BC} k \bar{c}_{i,j}^G \quad (2.24)$$

282 Symmetry boundary centre of the vial with biocomposite present – type “CS”

$$283 \quad \frac{\partial \bar{c}_{i,j}^G}{\partial \bar{t}} = - \left(\frac{\Delta r_0 r_0}{D_0} \right) a_{BC} k \bar{c}_{i,j}^G \quad (2.25)$$

284 Horizontal vial wall – type “WH”

$$285 \quad \frac{\partial \bar{c}_{i,j}^G}{\partial \bar{t}} = \frac{D_{CO_2}^G \Delta r_0}{D_0 r_0} \left(\frac{\bar{c}_{i+1,j}^G - 2\bar{c}_{i,j}^G + \bar{c}_{i-1,j}^G}{\Delta x^2} + \frac{2}{3} \left[\frac{\bar{c}_{i,j-1}^G - \bar{c}_{i,j}^G}{\Delta y^2} \right] \right) \quad (2.26)$$

286 Vertical vial wall – type “WV”

$$287 \quad \frac{\partial \bar{c}_{i,j}^G}{\partial \bar{t}} = \frac{D_{CO_2}^G \Delta r_0}{D_0 r_0} \left(\frac{2}{3} \left[\frac{\bar{c}_{i-1,j}^G - \bar{c}_{i,j}^G}{\Delta x^2} \right] + \frac{\bar{c}_{i,j+1}^G - 2\bar{c}_{i,j}^G + \bar{c}_{i,j-1}^G}{\Delta y^2} \right) \quad (2.27)$$

288 Corner vial wall – type “WC”

$$289 \quad \frac{\partial \bar{c}_{i,j}^G}{\partial \bar{t}} = \frac{2}{3} \frac{D_{CO_2}^G \Delta r_0}{D_0 r_0} \left(\frac{\bar{c}_{i-1,j}^G - \bar{c}_{i,j}^G}{\Delta x^2} + \frac{\bar{c}_{i,j-1}^G - \bar{c}_{i,j}^G}{\Delta y^2} \right) \quad (2.28)$$

290 Symmetry boundary (x-axis) at the wall – type “WSX”

$$291 \quad \frac{\partial \bar{c}_{i,j}^G}{\partial \bar{t}} = \frac{2}{3} \frac{D_{CO_2}^G \Delta r_0}{D_0 r_0} \left(\frac{\bar{c}_{i-1,j}^G - \bar{c}_{i,j}^G}{\Delta x^2} \right) \quad (2.29)$$

292 Symmetry boundary (y-axis) at the wall – type “WSY”

$$293 \quad \frac{\partial \bar{c}_{i,j}^G}{\partial \bar{t}} = \frac{2}{3} \frac{D_{CO_2}^G \Delta r_0}{D_0 r_0} \left(\frac{\bar{c}_{i,j-1}^G - \bar{c}_{i,j}^G}{\Delta y^2} \right) \quad (2.30)$$

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2.4 Solution

Fig. S7 Contour plot of the gas phase composition after 48 hours for CCAP 1479/1A biocomposite. Initial CO₂ concentration = 2.05 mols m⁻³ (5 mol% CO₂), light:dark ratio 16:8. The transfer coefficient for the cells in the suspension controls in the vial is 5 x 10⁻⁹ m s⁻¹. The characteristic time for diffusion across the vial is much shorter than the characteristic time for transfer through the biocomposite. There are three options for the diffusion of CO₂ through the latex coating.

1. The coating is a porous structure that CO₂ gas diffuses through. The DVS experiments gave the value of diffusivity for this as approximately 10⁻⁸ m² s⁻¹.
2. The coating is a porous structure saturated with water. In this case the diffusivity will be approximately that for CO₂ in water (10⁻⁹ m² s⁻¹).
3. The coating is a polymer film which the CO₂ has to dissolve into the film and diffuse through it. The diffusivity for CO₂ in polymer films is of the order of 10⁻¹³ m² s⁻¹.

The mass transfer coefficient for the biocomposite is given by the following equation.

$$a_c \left(\frac{1}{K a_c} \right)_{exp} = \frac{1}{K_{exp}} = \frac{1}{k_{cell}} + \frac{1}{k_{film}}$$

In this equation k_{cell} is the mass transfer coefficient for the cell in suspended culture. Using ranges of the surface area given in Table S4 we can calculate values for k_{film} .

$$k_{film} = \frac{D_{film}}{\Delta z_{film}}$$

315 The change in the mass transfer coefficient indicates that the CO₂ is having to diffuse through a solid polymer
316 film to reach the cells.

317 **Nomenclature**

318 a_c – specific surface area of cell interface ($\text{m}^2 \text{m}^{-3}$)

319 c^G – concentration of CO₂ in the gas phase (mols m^{-3}).

320 c^L – concentration of CO₂ in the liquid phase (mols m^{-3}).

321 c_0 – characteristic concentration (mols m^{-3}).

322 $D_{CO_2}^G$ – diffusivity of CO₂ in the gas phase ($\text{m}^2 \text{s}^{-1}$).

323 $D_{CO_2}^L$ – diffusivity of CO₂ in the liquid phase ($\text{m}^2 \text{s}^{-1}$).

324 D_0 – characteristic diffusivity ($\text{m}^2 \text{s}^{-1}$).

325 K – transfer coefficient across the cell membrane incorporating the rate of CO₂ consumption by the cell (m s^{-1}).

326 K_{ov} – mass transfer coefficient for CO₂ transport across the air-water interface (m s^{-1}).

327 N – number of cells in the gas layer.

328 M – number of cells in the liquid layer.

329 t – time (s).

330 z – axial distance in the vial (m).

331 z_0 – characteristic height (m).

332 t – characteristic time ($\Delta z_0 z_0 / D_0$) (s).

333 **Data**

334 Diffusivity of CO₂ in air = $1.6 \times 10^{-5} \text{ m}^2 \text{s}^{-1}$

335 Diffusivity of CO₂ in water = $1.92 \times 10^{-9} \text{ m}^2 \text{s}^{-1}$

336 Henry's constant for CO₂ in water at 25°C = $2938 \text{ m}^3 \text{mol}^{-1} \text{Pa}$

337 **Sample Vials**

338 Microalgae:

339 OD = 1.55 cm, height = 9.8 cm, wall thickness = 0.15 cm,

340 Volume = $\frac{1}{4} \pi (1.55 - 2 \times 0.15)^2 \times 9.8 = 12.0 \text{ ml}$

341 Cyanobacteria:

342 OD = 4.0 cm, height = 7.3 cm.

343 Height of straight section = 4.8 cm, wall thickness = 0.13 cm, base thickness = 0.28 cm.

344 Curved section is 1.3 cm tall and diameter goes from 4.0 cm to 1.4 cm.

- 345 Top straight section has wall thickness of 2.2 mm and height of 8 mm.
- 346 Volume of straight section at the bottom = $\frac{1}{4} \pi (4.0 - 2 \times 0.13)^2 \times (4.8 - 0.28) = 49.7 \text{ ml}$
- 347 Volume of curved section = $\frac{2}{3} \times \pi \times 0.5 \times (4.0 - 0.26) \times 1.3^2 = 6.6 \text{ ml}$
- 348 Volume of straight section at the top = $\frac{1}{4} \pi (1.4 - 2 \times 0.22)^2 \times 0.8 = 0.6 \text{ ml}$
- 349 Total volume = 56.9 ml
- 350 Equivalent vial with an OD of 4.0 cm, wall thickness = 0.13 cm and a height of 5.2 cm.
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Fig. S8 A schematic sequence of the process to immobilise microalgae on loofah sponge using latex binders for the continuous CO₂ test.

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388 **Table S4** Loss of photosynthetically active radiation (PAR) per unit depth (mm) for different loofah samples
 389 relative to ambient sunlight.

Sample	Thickness (mm) (mean $\pm$ Stdev)	PAR loss per unit depth (% loss/mm; mean $\pm$ Stdev)
Half loofah	13.01 $\pm$ 0.43	7.11 $\pm$ 0.02
Whole loofah (horizontal)	54.63 $\pm$ 0.72	1.79 $\pm$ 0.02
Whole loofah (vertical)	100.8 $\pm$ 0.27	0.97 $\pm$ 0.03

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Table S5 Photosynthetic conversion efficiency of the biocomposites as measured by bulk lipid content in the continuous CO₂ test.

Biocomposite	% lipid content per dry weight biomass (mean $\pm$ Stdev)	% photosynthetic conversion efficiency (mean $\pm$ Stdev)
<i>C. vulgaris</i> + S1	69.38 $\pm$ 1.27	0.036 $\pm$ 0.001
<i>C. vulgaris</i> + S3	51.96 $\pm$ 11.82	0.027 $\pm$ 0.006
<i>D. salina</i> + S1	36.1 $\pm$ 4.72	0.019 $\pm$ 0.002
<i>D. salina</i> + S11	66.22 $\pm$ 6.02	0.034 $\pm$ 0.003

Fig. S9 Schematic (top) and photograph (bottom) of the microalgae biocomposite rig for the continuous CO₂ fixation test.

Fig. S10 Toxicity testing using percentage growth normalised to suspension culture controls for (a) *C. vulgaris*, (b) *N. oculata*, and (c) *D. salina* with synthetic binders, and (d) *S. elongatus* CCAP 14791/A and (e) PCC 7942 with bio-based binders. *C. vulgaris*: Kruskal-Wallis, $H = 46.09$, $df = 13$, $P < 0.001$; *N. oculata*: $H = 47.55$, $df = 12$, $P < 0.001$; *D. salina*: $H = 37.41$, $df = 12$, $P < 0.001$; *S. elongatus* CCAP 14791/A: $H = 18.11$, $df = 6$, $P = 0.006$; Mann-Whitney, $U = 26$ ($n_{1,2} = 0.3326$), $P = 0.0304$ and PCC 7942: $H = 19.39$, $df = 6$, $P = 0.004$; Mann-Whitney, $U = 26$ ($n_{1,2} = 1.4571$), $P = 0.03$. Refer to Tables S1 and S2 for binder details.

Fig. S11 A representative 7-day toxicity test using *S. elongatus* CCAP 1479/1A with binders (a) B6 and (b) B4. A-C = binder + growth medium + cells, D = binder + growth medium, E = growth medium, F = growth medium + cells.

Fig. S12 Cumulative percentage of cells released from (a) *C. vulgaris*, (b) *N. oculata*, (c) *D. salina*, (d) *S. elongatus* CCAP 1479/1A and (e) PCC 7942 biocomposites. Determined as the cumulative release of cells; *C. vulgaris*: Kruskal-Wallis, $H = 49.03$, $df = 12$, $P < 0.001$, *D. salina*: $H = 16.57$, $df = 4$, $P = 0.002$, *N. oculata*: $H = 17.50$, $df = 4$, $P = 0.002$, *S. elongatus* CCAP 1479/1A: $H = 15.74$, $df = 5$, $P = 0.008$ and PCC 7942: $H = 15.62$, $df = 5$, $P = 0.008$. Refer to Tables S1 and S2 for binder details.

Fig. S13 SEM images of *S. elongatus* PCC 7942 biocomposites before and after adhesion testing. Binder B3 exhibiting poor cell retention (before = a&c, after = b&d), binder B1 exhibiting good cell retention (before = e&g, after = f&h). Magnification: 4000× (a, b, e, f) and 9000× (c, d, g, h).

Fig. S14 SEM images of the biocomposites after 8 weeks of net CO₂ fixation testing (semi-batch); (a-c) *C. vulgaris*, (d-f) *D. salina*, (g-i) *N. oculata*; Magnifications: 40×, 400× and 2500×.

Fig. S15 Development of representative *S. elongatus* biocomposites during the 8 weeks net CO₂ fixation test (semi-batch); (a) PCC 7942 + binder B5, (b) CCAP 1479/1A + binder B6.

Fig. S16 Light micrographs of *S. elongatus* PCC 7942 in: (a) BG11 growth medium (suspension control); and, (b) exposed to binder B6 at day 7 of the toxicity test. Control cells had a more rod-like morphology compared with the generally more coccoid morphology of the binder exposed cells.