Textile-based cyanobacteria biocomposites for potential environmental remediation applications

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Received: 16 September 2020 / Revised and accepted: 1 February 2021 / Published online: 24 February 2021
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Abstract

Microalgae and cyanobacteria are effective platforms for environmental remediation (phycoremediation), particularly of air and water. There is limited scope to deploy suspension cultures due to space, cost and maintenance challenges—driving an imperative towards biofilm-based treatment systems; however, these systems are ill-equipped for rapid and mobile deployment. In this study we explored the main technical challenges to developing cheap, accessible and low-maintenance engineered biofilm systems (biocomposites) comprising cyanobacteria (Synechococcus elongatus) immobilised to a range of textiles (n = 4) by natural or synthetic latex binders (n = 16), chitosan or shellac. Biocomposite viability (measured as net CO2 uptake) was assessed over 20 days in semi-batch trials. No maintenance was required during this period as the humidity within the reactor was sufficient to support metabolism. Two commercial natural latex binders (AURO 320 and 321) supported strong growth within the biocomposite, outperforming suspension controls. There was variation in textiles performance, with an 80/20 polyester-cotton blend performing most consistently. Biocomposite formulation was varied in terms of binder solids content and cell loading rate, with 5% solids and 2.5% cell loading the most effective combination. We demonstrate the technical feasibility of fabricating functional textile-based cyanobacteria biocomposites and discuss this within the context of developing decentralised wastewater treatment services.

Keywords Biocoating · Bioremediation · Carbon capture · Circular economy · Microalgae · Wastewater

Introduction

The growing global human population is placing increasing demands on water resources—demands which are unlikely to be met with current centralised treatment systems and practices (Lofrano and Brown 2010; Verstraete and Vlaeminck 2011). If the water industry is to keep pace, major technological innovations rather than incremental and piecemeal shifts in treatment practices are required (Thomas and Ford 2006; Tanner et al. 2018). The urgency for technology and process transition is heightened when viewed from a climate change context, with municipal wastewater treatment accounting for approximately 3% of global electricity consumption and 5% of non-CO2 greenhouse gas emissions (Li et al. 2015).

Decentralised (or point-source) systems present an alternative option, but to be effective they must be affordable, ensure both human and environmental safety, be user friendly (effectively zero maintenance), be fully compliant with effluent discharge regulations, and (ideally) should make a tangible contribution to the circular economy (Muñoz and Güeyssse 2006; Grant et al. 2012; Li et al. 2015; Cuellar-Bermudez et al. 2017; Oladoja 2017). Phycoremediation (the use of algae or cyanobacteria for environmental clean-up) is one approach that could deliver many of these requirements, with the capacity to combine wastewater and atmospheric remediation within a single treatment option, all the while generating biomass for bioprocessing (Olgün 2003; Rawat et al. 2011; Kumar et al. 2018; Ansari et al. 2019).

Due to their broad abiotic tolerances and metabolic flexibility, the use of cyanobacteria in wastewater treatment is well established (Oswald and Gotass 1957), forming an important part of mixed community activated sludge systems (Martins et al. 2011). However, targeted treatment processes based primarily on cyanobacteria are still under development. The very nature of suspension-based cultivation (generally conducted in high rate algae pond systems) remains one of the main
challenges facing the roll-out of microalgae and cyanobacteria treatment processes, with operational issues including poor batch-to-batch consistency, high economic cost of biomass separation, and contamination by non-target species (Christenson and Sims 2011), notwithstanding the substantial land requirement. Closed photobioreactors (PBRs) have been employed to mitigate abiotic variation and contamination (Mata et al. 2010; Tredici et al. 2015); however, capital and operational costs are prohibitive (Huang et al. 2017; Acién et al. 2018).

The need for suspension-based systems is being subverted with the advent of a range of biofilm reactors (Boelle et al. 2014; Zhuang et al. 2018; Peng et al. 2020a; Waqas et al. 2020; Yang et al. 2020), treating domestic (Gou et al. 2020), industrial (Johnson et al. 2018; Hillman and Sims 2020) and even marine wastewaters (Peng et al. 2020b). However, these systems still require sufficient operator competency to culture and maintain a metabolically active biofilm (David et al. 2015; Hamano et al. 2017). Iterations of these systems that reduce, or ideally eliminate, the need for regular culture maintenance is desirable in terms of process cost, accessibility and acceptability. Attempts in this direction include trialling phototropic granular biomass (photogranules) (Kumar and Venugopalan 2015; Trebuch et al. 2020) which, while promising in the context of a wastewater treatment plant, would not necessarily be suitable if the ambition for decentralisation extended as far as treatment systems for individual properties. Given such a scenario, a more radical vision of a biofilm reactor is needed. For instance, twinline systems using simple irrigation protocols show promise in cell retention and growth (Shi et al. 2007; Liu et al. 2013; Naumann et al. 2013; Shi et al. 2014) however, evaporation poses a problem that demands increased operator involvement.

Engineered, concentrated biofilms, also known as living biocomposites, are gaining traction in atmospheric and low flow wastewater bioremediation applications (Estrada et al. 2015; In-na et al. 2020). These biocomposites comprise living microbes immobilised within a semi-porous matrix (binder), forming a biocoating, which is then applied to a structural scaffold (de-Bashan and Bashan 2010), theoretically supporting greater biomass loading per unit area with minimal cell loss (Flickinger et al. 2007). Unlike natural biofilm or twin-layer systems, the use of immobilised biomass allows greater flexibility in reactor design, enabling increased reaction rates due to improved cell density, robustness, and greater reuse of biomass (Eroglu et al. 2015). Successful immobilisation has been demonstrated to improve O₂ production (an indicator of photosynthetic activity) between 7 and 10× compared with suspension cultures (Bernal et al. 2014). However, the efficacy of biocomposites can be affected by many factors during preparation, including, but not limited to the coating formulation, substrate properties and the extent of biomass loading (Flickinger et al. 2017) although novel fabrication methods show promise (Fidaleo et al. 2014; Bernal et al. 2017; Chen et al. 2020).

The current study drew inspiration from the EU H2020 Living Architecture project, which sought to develop highly distributed autonomous wastewater treatment systems for individual properties (Armstrong et al. 2017). We have extended the scope of the Living Architecture brief by evaluating some of the key technical constraints to immobilising cyanobacteria as biocomposites. Using two strains of *Synechococcus elongatus* (PCC 7942 and CCAP 1479/1A), we screened a range of binders formulated from latex, petroleum, shellac or chitosan, with four affordable and easily accessible textile scaffolds (a woven 80/20 polyester-cotton blend, a woven 100% bamboo, a non-woven 40/60 wool-polyester blend, and a non-woven 100% polyester). Binders were screened for toxicity and adhesive properties, with biocomposite viability determined by measuring net photosynthetic CO₂ uptake over a 20-day experimental period.

### Materials and methods

#### Cell cultivation

*Synechococcus elongatus* PCC 7942 was grown in Blue-Green Medium (BG11) (Stanier et al. 1971), and *S. elongatus* CCAP 1479/1A in Jaworski’s Medium (JM) (Šoštarić et al. 2012) without cyanocobalamin, thiamine HCl, and biotin, in 10 L polycarbonate (Nalgene) carboys with constant air supply at 18 °C ± 2 °C, and a 16L:8D photoperiod (mean irradiance of 35 μmol photons m⁻² s⁻¹) using 30 W daylight-type fluorescent tubes (Sylvania Luxline Plus, n = 6).

#### Binder adjustments

A selection of binders was screened for suitability for cyanobacteria immobilisation. Ten synthetic acrylic, styrene and polyurethane latex binders were selected from a library of proprietary and commercial binders, gifted by Prof. Michael Flickinger, North Carolina State University, USA, having previously been assessed for microbial immobilisation. Six plant-based binders (AURO Paint Company, UK and Germany) were similarly selected (Bernal et al. 2014; In-na et al. 2020). The initial percentage solids content of the liquid binders was determined by oven drying to a constant mass at 100 °C. The binders were adjusted to pH 7 using 0.1 M or 0.5 M acetic acid, or 0.1 M or 0.5 M ammonium hydroxide (Table 1). Shellac (orange, pure, flake, Fisher Scientific, CAS-9000-59-3, MDL no. 148309) was ground to a fine powder by mortar and pestle and dissolved in 90% ethanol at 50 °C. Chitosan (99.9%, ACROS Organics, CAS-9012-76-4, MDL no. MFCD00161512) was dissolved at 18 °C in 0.3 M acetic acid.
Table 1 Name, coding and available compositional details of the binders used in this study. Also shown are the binder adjustments required to make 1 mL of neutral pH binder. AA acetic acid, AH ammonium hydroxide, N/A not available

| Binder       | Code | Main components                                                                                                                                                                                                 | Initial % solids content | Binder volume (mL) | dH₂O to dilute to 5% (mL) | Neutraliser Final volume (mL) |
|--------------|------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------|--------------------|---------------------------|-------------------------------|
| AURO 160     | 160  | Castor oil, cellulose, colophony glycerol ester, drying agents, metal soaps, linseed oil, mineral pigments, silicic acid, surfactants made from rapeseed oil, water, xanthane                                             | 13.68                    | 0.364              | 0.564                     | 0.5M AA 0.073                 |
| AURO 251     | 251  | Castor oil, castor oil as amino soap, cellulose, colophony glycerol ester, drying agents, metal soaps, linseed oil, mineral fillers, sunflower oil, surfactants made of rapeseed and castor oils, water | 29.10                    | 0.172              | 0.802                     | 0.5M AA 0.026                 |
| AURO 261     | 261  | Castor oil, castor oil as amino soap, cellulose, colophony glycerol ester, drying agents, metal soaps, fatty acids, linseed oil, mineral fillers, silicic acid, sunflower oil, surfactants made of rapeseed oil, water | 19.52                    | 0.257              | 0.718                     | 0.5M AA 0.026                 |
| AURO 320     | 320  | Mineral fillers, water, Replebin®, titanium dioxide, cellulose, surfactants made of rapeseed-, castor oil, ammonia and thiazoles                                                                               | 52.57                    | 0.095              | 0.811                     | 0.5M AA 0.093                 |
| AURO 321     | 321  | Mineral fillers, water, Replebin®, titanium dioxide, cellulose, surfactants made of rapeseed-, castor oil, ammonia and thiazoles                                                                               | 52.85                    | 0.094              | 0.890                     | 0.1M AA 0.016                 |
| AURO 379     | 379  | Water, shellac (as ammonia soap), alcohol, xanthane, walnut oil, rosemary oil, lavender oil                                                                                                                  | 9.01                     | 0.545              | 0.273                     | 0.1M AA 0.182                 |
| Rhoplex SG-10M | 1    | Butyl acrylate/Methyl methacrylate                                                                                                                                                                           | 51.30                    | 0.098              | 0.891                     | 0.1M AA 0.011                 |
| Interkem A510 | 2    | Acrylic polymer                                                                                                                                                                                               | 25.08                    | 0.201              | 0.781                     | 0.1M AA 0.018                 |
| Baycusan C-1000 | 3    | Polyester polyurethane                                                                                                                                                                                     | 52.60                    | 0.096              | 0.903                     | 0.1M AA 0.001                 |
| Baycusan C-1004 | 4    | Polyester polyurethane                                                                                                                                                                                     | 42.68                    | 0.119              | 0.858                     | 0.5M AH 0.024                 |
| Nuplex Setaqua 6776 | 5    | Acrylic polymer                                                                                                                                                                                               | 42.77                    | 0.118              | 0.835                     | 0.1M AA 0.047                 |
| Rhoplex SF-012 | 6    | Acrylic polymer                                                                                                                                                                                               | 42.74                    | 0.117              | 0.883                     | - 0.000                      |
| Rhoplex SF-3122 | 7    | Acrylic polymer                                                                                                                                                                                               | 64.36                    | 0.079              | 0.909                     | 0.1M AA 0.012                 |
| JP 912       | 8    | Acrylic polymer                                                                                                                                                                                               | 51.12                    | 0.098              | 0.882                     | 0.1M AH 0.020                 |
| PD-0413      | 9    | N/A                                                                                                                                                                                                         | 80.37                    | 0.063              | 0.931                     | 0.1M AA 0.006                 |
| HB 3691-M    | 10   | Polyester polyurethane                                                                                                                                                                                      | 48.42                    | 0.105              | 0.848                     | 0.1M AA 0.047                 |

*AURO 320 has since been discontinued by the manufacturer
Toxicity testing

The binders were initially screened for cell growth impacts. One millilitre of binder was added to 1 mL of 7-10 days old culture (n=3) in a multiwell plate. In separate wells, 1 mL of binder and 1 mL of growth medium were combined to screen for interactions. One millilitre of cells diluted with 1 mL of sterile de-ionised water (dH2O) was run as a baseline for cell growth. Each treatment replicate was mixed daily by forward and reverse pipetting for 7 days, when cell density was determined using an improved Neubauer haemocytometer with a Leica DMi 8 microscope with bright field contrast at 400× magnification and viewed using LasX software. The most suitable binder for each strain was carried forward for subsequent adhesion testing, wherein the range of percentage solids contents was expanded to 2.5, 7.5, and 10% (>10% solids inhibits gas exchange (Umar 2018)).

Adhesion testing

The textile substrates were as follows: 1) a woven 80/20 polyester-cotton blend (cotton) purchased from Aow RungRuang Co. Ltd, Bangkok, Thailand; 2) a woven 100% bamboo (bamboo) was purchased from WBL Fabrics, UK; 3) a non-woven 40/60 wool-polyester blend (wool blend); and 4) a non-woven 100% polyester (polyester) were purchased from the Wool Felt Company, UK. The polyester-cotton blend was selected as it exhibited high levels of cyanobacteria attachment in a separate study (In-na et al. unpublished). The textiles were autoclaved, then dried at 105 °C for 3 h and stored in a silica gel desiccator until use. When required, the textiles were cut into 0.5 x 0.5 cm² pieces. Cyanobacteria were centrifuged at 1720×g for 30 min at 20 °C. If sufficient biomass was not recovered, the supernatant was removed and additional culture was added to the pellet and the centrifugation step was repeated. The cyanobacteria and binders were combined in sterile Eppendorf tubes, first by gentle pipetting and then by vortex mixing. A range of cell-binder formulations (biocoatings) were produced at 1, 2.5, 5, and 10% (v/v) cell content to understand the binder’s robustness. Once combined, 100 μL of each biocoating was pipetted onto the textiles to form biocomposites. In a separate Eppendorf tube, the equivalent volume of culture was added to the same volume of dH2O and counted using a Neubauer haemocytometer to estimate biocoating cell density and hence biocomposite cell loading. The biocomposites were dried in darkness at 20±2 °C for 24 h, then added to 1 mL of growth medium in well plates wrapped in aluminium foil to prevent cell division which would cause an overestimate of the number of cells lost from the biocomposite. The plates were placed on a two-dimensional orbital shaker (Techlifer orbital rotator shaker lab UPC 789458170564), set to 80 rpm. Biocomposites were moved to a new well of fresh medium after 1, 24, and 48 h with the total time spent in the medium being 72 h, allowing for cumulative cell release to be calculated using a Neubauer haemocytometer.

Scanning electron microscopy

The biocomposites were imaged using scanning electron microscopy following adhesion testing. Samples were dried at 105 °C for 3 h, stored in a desiccator before being attached to 12 mm diameter pin stubs using carbon tape and imaged using a Hitachi TM 3000 SEM with a backscattered electrons system. All biocomposites were observed in two or more randomised locations using a 5 or 15 kV accelerating voltage.

CO2 uptake

Informed by the toxicity and adhesion tests, biocomposites were taken forward for CO2 uptake tests. Samples were prepared as described for adhesion testing, but the textiles were cut into 1 × 5-cm strips. Formulations were pipetted 1 cm from the top of the strip so the growth medium could only reach cells by capillary action. Five millilitre of sterile growth medium was pipetted into a 50-mL sterile, clear Wheaton glass serum bottle. Biocomposite samples were then placed into the bottle and suspended using 0.15 mm sterile nylon thread to prevent the cells from being submersed. The bottles were sealed using a rubber butyl stopper and a crimped aluminium cap. Each bottle was flushed with 45 mL of 5% CO2-enriched air using a hypodermic needle to pierce the rubber stopper without breaking the seal. Samples containing the equivalent number of cells in suspension were placed in sealed bottles as controls. The samples were exposed to a mean irradiance of 35 μmol photons m⁻² s⁻¹, and a 16L:8D photoperiod using 30 W daylight-type fluorescent tubes (Sylvania Luxline Plus, n = 6) at 18 °C ± 2 °C. Every 2 days, a sample of the headspace was withdrawn using a hypodermic needle and an air-tight syringe and the percentage CO2 content was analysed using a G100 GEOTech CO2 meter. Bottles were then re-flushed with 5% CO2-enriched air. The moles of CO2 fixed by the cells were calculated using equation 1. This was repeated for 20 days and a cumulative total of fixed CO2 was calculated.

\[
\text{fixed CO2 (mol)} = \frac{(5.00\% - \% \text{CO}_2 \text{ recorded}) \times 45 \times 10^{-3} (L) \times \text{system pressure (atm)}}{0.082 (L \text{ atm mol}^{-1} \text{ K}^{-1}) \times \text{system temperature (K)}}
\]  

(1)

Biocomposites comprising 5% solids binder with 2.5% cell content, 10% solids binder with 2.5% cell content, and 5% solids with 10% cell content were tested following initial screening trials (data not shown). Additionally, a 5% solid binder with 2.5% cell content cotton biocomposite was tested with an artificial urine developed by Brooks and Keevil (2003) (1 g peptone L37, 0.005 g yeast extract, 0.1 g lactic acid).
acid, 0.4 g citric acid, 2.1 g NaHCO₃, 10 g urea, 0.07 g uric acid, 0.8 g creatinine, 0.37 g CaCl₂·2H₂O, 5.2 g NaCl₂, 0.0012 g FeSO₄·7H₂O, 0.49 g MgSO₄·7H₂O, 3.2 g Na₂SO₄·10H₂O, 0.95 g KH₂PO₄, 1.2 g K₂HPO₄, 1.3 g NH₄Cl, to 1 L dH₂O) rather than a conventional growth medium to better reflect a wastewater scenario.

Statistical analysis

Statistical analyses were conducted using GraphPad Prism 8. Data were tested for normal distribution (Shapiro-Wilk test). For toxicity data, equality of variance was determined using the Brown-Forsythe test and one-way ANOVA with Tukey post hoc testing. For adhesion data, two-way ANOVA was used with Tukey post hoc testing. Sphericity was assumed due to measurements as the experimental design was based on matching, not repeated measures (Maxwell et al. 2017). For CO₂ uptake, repeated measures two-way ANOVA with Tukey post hoc testing was conducted. For the adhesion and CO₂ data, sphericity was violated; therefore, a Geisser-Greenhouse correction was performed (Greenhouse and Geisser 1959).

Results

Binder toxicity

The toxicity data for both *S. elongatus* strains were normally distributed (Shapiro-Wilk test, *P* > 0.05), with significant differences between control cultures and 5% solids AURO binders (*S. elongatus* PCC 7942: ANOVA, *F*₆,₁₇ = 483.3, *P* < 0.001; *S. elongatus* CCAP 1479/1A: ANOVA, *F*₆,₁₄ = 70.67, *P* < 0.001), synthetic binders (*S. elongatus* PCC 7942: ANOVA, *F*₁₀,₃₅ = 10.3, *P* < 0.001; *S. elongatus* CCAP 1479/1A: ANOVA, *F*₁₀,₂₂ = 82.09, *P* < 0.001), and natural binders (shellac and chitosan) (*S. elongatus* PCC 7942: ANOVA, *F*₂,₉ = 128.7, *P* < 0.001; *S. elongatus* CCAP 1479/1A: ANOVA, *F*₂,₆ = 224.6, *P* < 0.001).

Of the AURO binders, only AURO 320 and 321 supported significantly improved growth, whereas AURO 160, 251, 261 and 379 returned cell densities significantly lower than controls (*P* < 0.05); therefore, in this context, they were classed as toxic (Fig. 1). On this basis, only AURO 320 and 321 were considered for follow-on CO₂ fixation trials. None of the
synthetic binders supported improved growth relative to controls with a singular exception of binder-9 with *S. elongatus* CCAP 1479/1A; however, this was not deemed to be a sufficiently large growth improvement to justify automatic inclusion in subsequent CO₂ trials. The shellac and chitosan treatments had significantly decreased cell densities for both strains, with shellac killing the cultures.

**Effects of AURO binder solids content**

There was a significant increase in growth with respect to controls for *S. elongatus* PCC 7942 with AURO 320 (ANOVA, $F_{4,13} = 23.06, P < 0.001$), with the best growth support by the 2.5% solids treatment ($P < 0.001$). Similarly for *S. elongatus* CCAP 1479/1A with AURO 321 (ANOVA, $F_{4,10} = 5.182, P = 0.016$) (Fig. 2), although in this case the best growth was with the 7.5% solids treatment ($P = 0.001$).

**Adhesion**

Scanning electron micrographs of representative *S. elongatus* PCC 7942 biocomposites are presented in Fig. 3, showing variation in biocoating integrity. Figure 3a illustrates the densely packed nature of the biocoating, but also shows that the biocoatings cannot be regarded as monolayers. Equally, poorly coated areas of the fibres are seen (Fig. 3b and c), as well as a more complex 3D architecture where the pore space between fibres has allowed the biocoating to penetrate further.
into the textile structure (Fig. 3d). Significant interactions between textile type and binder solids content affected cell retention when a 1% cell loading was used (two-way ANOVA: $F_{12} = 4.267$, $P < 0.001$), with textile type (two-way ANOVA: $F_{3} = 15.93$, $P < 0.001$) and solids content (two-way ANOVA: $F_{4} = 31.38$, $P < 0.001$) having significant effects. Cells’ retention was not significantly different for the bamboo at any binder solids content, but the other textiles experienced significant losses (Fig. 4), particularly for higher solids contents, with wool and polyester having the poorest cell retention properties.

When a 2.5% cell loading was used there was no significant interaction between textile type and binder solids content (two-way ANOVA: $F_{12} = 1.732$, $P = 0.096$). Cell retention was significantly reduced for all textiles at any binder solids content relative to the control (Fig. 4). For cotton and bamboo, there was a general trend of improved cell retention with increasing solids content, but this was not evident for either the wool blend or the polyester.

There was a significant interaction between textile and binder (two-way ANOVA: $F_{12} = 4.365$, $P = 0.001$) when 5% cell loading was used, with textile type (two-way ANOVA: $F_{3} = 11.40$, $P < 0.001$) and binder solids content (two-way ANOVA: $F_{4} = 16.16$, $P < 0.001$) having significant effects.

There was no significant release for either cotton or bamboo at any solids content (Fig. 4); however, the wool and polyester textiles experienced significant cell loss at 7.5 and 10% solids.

There was also a significant interaction between textile and binder at the highest cell loading (10%) (two-way ANOVA: $F_{12} = 5.052$, $P < 0.001$), with textile (two-way ANOVA: $F_{3} = 14.26$, $P < 0.001$) and binder solids (two-way ANOVA: $F_{4} = 27.12$, $P < 0.001$) both significant factors. The bamboo had no significant cell loss and the cotton only had significant loss at 2.5% solids, whereas the wool blend had significant release at 7.5 and 10% solids content and the polyester at 2.5, 7.5, and 10% solids content.

In the equivalent adhesion tests with *S. elongatus* CCAP 1479/1A, there was also a significant interaction between textile and binder solids content at a cell loading of 1% (two-way ANOVA: $F_{12} = 14.3$, $P < 0.001$). Textile (two-way ANOVA: $F_{3} = 48.55$, $P < 0.001$) and binder solids content (two-way ANOVA: $F_{4} = 151.7$, $P < 0.001$) were significant factors. All textiles experienced some cell loss although the bamboo was unaffected by the binder solids content. The other textiles experienced increased cell loss at high binder solids content (either 7.5 or 10% solids) (Fig. 5). The wool blend and the polyester treatments performed very poorly at higher solids content, in some instances (polyester 7.5 and 10%) causing all cells to be released.
A markedly different pattern was observed at a cell loading of 2.5%. There was no significant interaction between textile and binder solids (two-way ANOVA: $F_{12} = 1.533, P = 0.153$). Whereas all treatments did release cells, this was either not worsened at higher solids content or, in the case of cotton, cell retention tended to improve.

The 5 and 10% cell loading treatments returned very similar patterns in terms of cell release. In both cases, there were significant interactions between textile and binder solids (5% cell loading—two-way ANOVA: $F_{12} = 3.307, P = 0.002$; 10% cell loading—two-way ANOVA: $F_{12} = 5.708, P < 0.001$). Textile was a significant factor (5% cell loading—two-way ANOVA: $F_{3} = 9.607, P < 0.001$; 10% cell loading—two-way ANOVA: $F_{3} = 13.99, P < 0.001$) as was binder solids content (5% cell loading—two-way ANOVA: $F_{4} = 20.99, P < 0.001$; 10% cell loading—two-way ANOVA: $F_{4} = 21.74, P < 0.001$). The cotton and bamboo textiles had no significant cell losses at any binder solids content at either cell loading levels. The performance of the wool blend was almost identical between the cell loading rates, with only the 7.5 and 10% binder solids losing cells. Whereas the polyester textile did tend to lose cells at higher binder solids, this loss was not quite as high at the 7.5% cell loading level.

**CO₂ uptake for S. elongatus PCC 7942 biocomposites**

For biocomposites with 5% solids content and 2.5% cell loading, there was a significant interaction between textile and time (days) in terms of CO₂ consumption (two-way ANOVA: $F_{40} = 6.871, P < .0001$), with textile (two-way ANOVA: $F_{4} = 9.192, P < 0.001$) and time (two-way ANOVA: $F_{10} = 909.4, P = 0.016$) significant factors for *S. elongatus* PCC 7942 (Fig. 6a). Cotton and polyester had significantly more CO₂ uptake per cell than the suspension control from days 2 and 6 respectively. However, there was no significant difference in CO₂ uptake per cell between textiles within each time point.
There was a significant interaction between textile and time in terms of CO$_2$ uptake for *S. elongatus* PCC 7942 with 10% solids content AURO 320 (two-way ANOVA: $F_{40} = 6.871, P < 0.001$), with textile (two-way ANOVA: $F_d = 9.192, P = 0.002$) and time (two-way ANOVA: $F_{10} = 909.4, P < 0.001$) significant factors (Fig. 6b). The CO$_2$ uptake of the suspension control was significantly higher than the biocomposites at each time point. The bamboo was the poorest performing biocomposite.

For *S. elongatus* PCC 7942 biocomposites with 5% solids content and 10% cell loading, textile and time had significant interactions (two-way ANOVA: $F_{40} = 14.68, P < 0.001$), with both time (two-way ANOVA: $F_{10} = 631.8, P < 0.001$) and textile (two-way ANOVA: $F_d = 17.98, P = 0.001$) significant factors (Fig. 6c). From day eight onwards, cells immobilised to cotton or bamboo had significantly lower cumulative CO$_2$ uptake than the suspension control, whereas the wool and polyester were not significantly different to the control. The CO$_2$ uptake of all treatments (including the controls) was markedly reduced compared with preceding trials.

For the *S. elongatus* PCC 7942 biocomposites fabricated with 5% solids content and 2.5% cell loading, there were significant interactions between textile, time and growth media (two-way ANOVA: $F_{40} = 49.88, P < 0.001$), with time (two-way ANOVA: $F_{10} = 1053.00, P < 0.001$) and textile and media (two-way ANOVA: $F_d = 77.98, P < 0.001$) all significant factors (Fig. 6d). From day four onwards, BG11 treatments had greater cumulative CO$_2$ uptake than their respective suspension and cotton controls. With artificial urine, cumulative CO$_2$ uptake was not significantly different either in suspension or immobilised. From day six, cumulative CO$_2$ uptake with BG11 was significantly increased with respect to all other treatments regardless of immobilisation state. Suspension cultures failed to significantly increase cumulative CO$_2$ after day four in artificial urine and day six in BG11, in contrast to immobilised samples which had significant increases in cumulative CO$_2$ at all time points until day 16.

**CO$_2$ uptake for *S. elongatus* CCAP 1479/1A biocomposites**

There was a significant interaction between textile and time for biocomposites made with 5% solids content and 2.5% cell loading (two-way ANOVA: $F_{40} = 5.325, P < 0.001$), with time (two-way ANOVA: $F_{10} = 646.7, P < 0.001$) and textile (two-way ANOVA: $F_d = 4.987, P = 0.018$) significant factors. From days 4–18, there were no significant differences in cumulative CO$_2$ uptake between suspended and immobilised cultures, with the exception of the wool blend on day 20 (Fig. 7a). Over time, only the wool and polyester made significant increases in cumulative CO$_2$ uptake.

For biocomposites fabricated with 10% solids and a 2.5% cell loading (Fig. 7b), there was a significant interaction between textile and time (two-way ANOVA: $F_{40} = 6.249, P < 0.001$), with time (two-way ANOVA: $F_{10} = 1426.00, P < 0.001$) and textile (two-way ANOVA: $F_d = 8.404, P = 0.003$) significant factors. There was no significant difference in cumulative CO$_2$ uptake between textiles. From days 8 and 10 onwards, the bamboo and polyester had significantly lower cumulative CO$_2$ uptake than the suspension control.

There was a significant interaction between textile and time (two-way ANOVA: $F_{40} = 1.564, P = 0.038$) with time (two-way ANOVA: $F_{10} = 536.0, P < 0.001$) similar for all textiles. Additionally, there was no significant difference in cumulative CO$_2$ uptake between the biocomposites made with 5% solids content and 10% cell loading (Fig. 7c). Only the cotton biocomposite did not have significantly different cumulative CO$_2$ uptake compared to the suspension control. However, from day eight onwards, there was no significant difference in cumulative CO$_2$ uptake between the suspension control or textiles.

In the final experiment, with biocomposites made with 5% solids content and 2.5% cell loading, there were significant interactions between textile, time and the growth media (two-way ANOVA: $F_{40} = 8.613, df = 30, P < 0.001$), with time (two-way ANOVA: $F_{10} = 326.1, P < 0.001$) and textile and media (two-way ANOVA: $F_d = 13.68, P = 0.016$) significant factors (Fig. 7d). The use of artificial urine with the immobilised biocomposites did not cause a significant change in cumulative CO$_2$ uptake compared to the equivalent JM treatment until day 18. The impact of artificial urine on the suspension or immobilised samples was less clear over time. The suspended samples had significantly greater cumulative CO$_2$ uptake on days 4 and 8. Biocomposites exposed to either artificial urine or JM had significantly increases at each subsequent measurement over the full experimental period, but the suspension treatment did not make significant increases on after days 2 and 10 respectively.

**Discussion**

The objective of this study was to investigate some of the main technical steps required to develop robust, low cost, low maintenance living cyanobacterial biocomposite systems that could be deployed for a range of phycoremediation applications. As a system, the biocomposites are inherently simple, comprising three core elements: a scaffold to provide the physical means of supporting the structure (in this study this role was fulfilled using a range of commercially sourced textiles), a binder (ideally
porous) to secure the cells to the scaffold while allowing for the diffusion of water and gases across the thin polymer film, and an appropriate microorganism. Textiles were chosen as they are cheap, easily obtained and could extend the technical life of the textile product if discarded fabrics are repurposed as biocomposites. Textiles also have the added benefit of being highly porous scaffolds with a large surface area for biocoating adhesion. Their physical flexibility would also allow textile biocomposites to be deployed in a range of physical spaces and could deliver remediation using both dead-end and tangential flow (in addition to the capillary action and wicking demonstrated here), although the latter would be favoured.

We chose to test a range of textiles, ranging from 100% natural fibres (bamboo) to fully synthetic (100% polyester), and incorporating two textile blends (a 40/60 wool-polyester blend and an 80/20 polyester-cotton blend); however, other scaffolds have been tested (Akhtar et al. 2004; Bernal et al. 2014; Eroglu et al. 2015). Polyester was chosen as it is ubiquitous throughout the textiles industry and biocomposites could offer an additional recycled product. Furthermore, we have separately demonstrated that polyester is amenable to conversion to cyanobacterial biocomposites for carbon capture applications, particularly the 80/20 blend used here (In-na et al. unpublished); although we acknowledge the need to drastically reduce plastics use and subsequent release into the environment (Barnes et al. 2009). The wool blend was chosen against the backdrop of a collapse in the wool price (currently trading at a 6-year low, 18 August, 2020, www.tradingeconomics.com), with many UK wool producers having to destroy their fleeces. At the opposite end of the scale we chose bamboo, and despite it being an expensive material, the growing demand for bamboo clothing is driving increased production that should, in time, reduce wholesale prices.

Binder choice was guided by prior experience. A range of synthetic binders were screened, and although we previously documented toxicity issues when used in combination with eukaryote microalgae (In-na et al. 2020), they had not been assayed against cyanobacteria. However, the outcomes were very similar, with all binders proving toxic with the exception of PD-0413 (binder 9). Nonetheless, we elected not to promote this binder for further testing due to its unknown properties and focused more effort on the AURO latex coatings, which are more accessible.

AURO coatings are formulated from exclusively natural ingredients and are hypoallergenic; they were chosen on the premise that they should have lower toxicity to the cyanobacteria. This assumption was not entirely justified as four of the six AURO binders returned cell densities below the controls. Without a detailed reverse engineering of the formulations, it is not possible to identify where the issues lie; however, comparing the published constituents reveals that binders 160, 251, and 261 all contain metal soaps (to promote coating drying) which are water-insoluble compounds comprising alkaline earth or heavy metals (the manufacturer does not declare which metals are involved) contained within carboxylic acids (Robinet and Corbeil- a 2003; Noble 2019). We also cannot discount the possibility that the impact on cell growth was not due to the polymer film being impermeable, thereby impeding gas and water exchange. Binder 379, which has shellac as a constituent of the formulation, also supported poor growth. Given that the shellac-only treatment killed all of the cyanobacteria, it is not unreasonable to deduce that the shellac had a role in the poor performance of this binder.

The two AURO emulsion coatings (320 and 321) successfully supported strong cyanobacteria growth (with some variation between the strains). These observations corroborate our previous findings with these coatings when used in combination with a loofah sponge scaffold (In-na et al. 2020), consistently outperforming suspension controls, often by orders of magnitude. Other than not containing metal soaps or shellac, the reasons for such consistent growth promotion remain speculative. Both binders list ammonia as part of the formulation, which potentially could be exploited by S. elongates as an additional nitrogen source (Ludwig and Bryant 2012), although nitrogen limitation is unlikely to have been an issue in the short (72 hour) toxicity tests. The other notable differences are the inclusion of Replebin® (In-na et al. 2020) (a plant alcohol ester with organic acids) as a proprietary ingredient, and titanium dioxide as a pigment. We have no further details on the chemical composition of Replebin® and speculation would be unhelpful; however, a potential role—albeit paradoxi
cal—for TiO2 cannot be discounted. TiO2 is increasingly used in photochemistry based water treatment to kill cyanobacteria and destroy their cyanotoxins (He et al. 2020; Pestana et al. 2020); yet a recent study has found that colloidal TiO2 and cyanobacteria extracellular polymeric substances (EPS) interact, promoting colloidal stability, reducing photochemical damage (Xu et al. 2020), and potentially enhancing the adsorption of organic molecules, particularly those with phosphate and nitrogen moieties. Further investigation into the functional aspects of the AURO 320 and 321 binders is clearly warranted.

Despite previous studies successfully immobilising microalgae and cyanobacteria in chitosan (Aguilar-May et al. 2007; Aguilar-May and Del Pilar Sánchez-Saavedra 2009; Eroglu et al. 2015), there was a significant reduction in growth during toxicity testing, potentially due to suboptimal acetic acid pH of 3.13 experienced during the curing process. A more structure approach to developing chitosan within a biocomposite architecture would probably address these issues.

The second determinant of a successful binder is its capacity to retain the cells once the biocoating is formed. SEM imaging revealed variation in the coverage of the textile fibres by AURO 320 and 321, and that the manual application approach used here does not deliver a cellular monolayer with the topographically complex textiles. The polyester-cotton and bamboo textiles were both woven; the diameter and spacing of the weave affects the inter-yarn pore size, subsequently affecting the swelling ability of the textile as the liquid flows between the pores, with tighter weaves allowing less swelling.
slippage, the CO_2 uptake was reduced compared to the sus-

tainable moisture which could contribute to biocoating failure.

swelling of rubber latex of up to 20% under exposure to con-

'through evaporation and wicking, with the extent of wicking

coating latex onto porous substrates, the water can leave

CO_2 uptake over a 20-day semi-batch trial, with reference to

2000).

cyst viscosity and prevented levelling of the film (Desjumaux et al.

improved stability and abrasio n resistance (Dornyi et al.

utilised in the cotton industry to penetrate the cellulose by

and helped create more even films with improved adhesive

properties.

Interestingly, the ammonia in the AURO binders may have

physically affected the cotton and bamboo. Ammonium is

utilised in the cotton industry to penetrate the cellulose by

breaking hydrogen bonds, with the resulting fibres having

improved stability and abrasion resistance (Dornyi et al.

2008). Additionally, the increased cell density in the adhesion

test may also have affected film formation by increasing vis-

cosity and prevented levelling of the film (Desjumaux et al.

2000).

The viability of the biocomposites was determined by net

CO_2 uptake over a 20-day semi-batch trial, with reference to

the equivalent cell density in suspension culture. The biocomposites with 5% solids content and 2.5% cell loading

performed well, easily surpassing the controls, although over time there was some slippage of the biocoating from the wool

blend and polyester biocomposites into the liquid media pool.

Latex films can begin to disintegrate after just 10 days, pri-
marily due to photodegradation but also from microbial activ-

ity (Lambert et al. 2013). Furthermore, there is potential for

swelling of rubber latex of up to 20% under exposure to con-

tinuous moisture which could contribute to biocoating failure

and subsequent release of cells (Cesar et al. 2020).

When the solids content was increased to 10% to prevent

slippage, the CO_2 uptake was reduced compared to the sus-

pension controls. The increased thickness of the binder and

the reduced number of pores will have reduced light penetra-

tion, gas and nutrient exchange through mass transfer limita-

tion (Pires et al. 2013; Miranda et al. 2017), which we con-

firmed in our previous work (In-na et al. 2020). Future iter-

ations of these biocomposites should focus on maximising pore

number and size (i.e., permeability) without compromising

the structural integrity of the biocoating or by exceeding the

cell size of the immobilised organism. A number of options

exist to achieve this, including incorporating water soluble

fillers within the binder (Lyngberg et al. 2001) and, at a more

technical level, including halloysite nano clays (Chen et al.

2020).

To increase CO_2 uptake, more biomass was loaded into the

system; however, the CO_2 uptake per cell was significantly

reduced when compared with the 2.5% biocoatings.

Increasing cell loading in immobilised systems is not neces-

sarily a panacea for poor performance, as demonstrated in

alginate immobilised systems (Chevalier and de la Noüe

1985; Lau et al. 1997; Hameed 2007). In scenarios where

resource limitation will become an issue (such as the single

addition of nutrients in the current study), by increasing cell

loading you only exacerbate resource competition. Whereas

we did not specifically measure nutrient levels, it is reasonable

to assume a degree of limitation given the 20 day duration

without nutrient renewal. This would have reduced pigment

synthesis, compromised photosynthetic efficiency, and there-

fore reduced CO_2 fixation (Ruan et al. 2018). In the context of

wastewater remediation, this may be less of an issue as nutri-

tent deficiency is less likely outside of static systems as tested

here.

To better reflect the performance of the biocomposites

when exposed to effluent, the defined growth media were

replaced with a basic artificial urine. Both S. elongatus strains

had reduced CO_2 uptake with the artificial urine. Most
cyanobacteria have one or more genes that transport and

catabolise urea to ammonia and CO_2 which can then be used

for metabolism (Veaudor et al. 2019). Li and co-workers com-
pared the proteomic response of Synechococcus sp. WH8102

when grown on either nitrate or urea, with the urea treatments

having greater RuBisCO activity but reduced carbonic

anhydrase activity, suggesting a higher carboxylation efficien-
ty which reduces demand for CO_2 (Li et al. 2019b). The

artificial urine had sufficient phosphate; therefore, deviation

from stoichiometric balance does not explain the observations.

Once optimised biocomposites are developed, there is need

for further optimisation based on real effluent.

Aside from the materials components of the biocomposites,
careful selection (and potentially adaptation) of the

immobilised organism is also needed. We have observed sub-

stantive differences in performance between two strains of

the same species, with S. elongatus PCC 7942 better equipped for

immobilised existence. We documented similar variation (al-

beit with S. elongatus CCAP 1479/1A delivering the better

results) using loofah sponge-based biocomposites for carbon

capture. For biocomposites to reach their potential, there is

an imperative to incorporate species with established

phycoremediation pedigree in addition to convenient labora-

tory strains. In conjunction with this, there is a need for individ-

ual, informed tailoring of biocomposites and photobioreactor
design for their specific utilisations which can be determined

using a model-based approach (Li et al. 2019a).
Conclusion

We set out to explore the technical constraints to fabricating textile-based cyanobacteria biocomposites that could be deployed for a range of environmental remediation applications. In particular, these approaches (pending further optimisation) could become an effective treatment option for highly distributed wastewater treatment infrastructure, potentially within the context of individual properties. This study drew inspiration from the EU H2020 project Living Architecture (Armstrong et al. 2017), which had the goal of developing similar highly distributed wastewater treatment infrastructure; however, the Living Architecture technology is deeply embedded within the mindset of suspension-based treatment systems. We sought to circumvent this approach by developing engineered immobilised biocomposites that would accelerate the maturation of the Living Architecture vision and lead to the miniaturisation (i.e., intensification) of the Living Architecture infrastructure, which will be vital if it is to deliver on its promise.

Acknowledgements We thank Dr Abbas Umar for help with laboratory training, Peter McParlin, Charlotte Anderson and Matthew Walker for technical assistance, Dr Juan Nogales for the donation of S. elongatus PCC 7942, Michael Foster for assistance with SEM imaging, and we are especially grateful to Prof. Michael Flickinger for making his synthetic latex binders available for testing.

Author contribution The research was conceived by all authors, data collection and analysis were conducted by Rachel Hart and Pichaya In-na, the manuscript was written by Rachel Hart and Gary Caldwell and edited and approved by Pichaya In-na, Maxim Kapralov, and Jonathan Lee.

Funding PI was supported by a Newcastle University Doctoral Scholarship.

Data availability At request to the corresponding author.

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