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PAPER

Biological, physical and morphological factors for the programming of a novel microbial hygromorphic material

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Keywords: hygromorph, active material, bacterial spore, passive humidity regulation

OPEN ACCESS

RECEIVED
30 November 2023

REVISED
8 February 2024

ACCEPTED FOR PUBLICATION
3 April 2024

PUBLISHED
18 April 2024

Abstract

The urgency for energy efficient, responsive architectures has propelled smart material development to the forefront of scientific and architectural research. This paper explores biological, physical, and morphological factors influencing the programming of a novel microbial-based smart hybrid material which is responsive to changes in environmental humidity. Hygromorphs respond passively, without energy input, by expanding in high humidity and contracting in low humidity. *Bacillus subtilis* develops environmentally robust, hygromorphic spores which may be harnessed within a bilayer to generate a deflection response with potential for programmability. The bacterial spore-based hygromorph biocomposites (HBCs) were developed and aggregated to enable them to open and close apertures and demonstrate programmable responses to changes in environmental humidity. This study spans many fields including microbiology, materials science, design, fabrication and architectural technology, working at multiple scales from single cells to 'bench-top' prototype.

Exploration of biological factors at cellular and ultracellular levels enabled optimisation of growth and sporulation conditions to biologically preprogramme optimum spore hygromorphic response and yield. Material explorations revealed physical factors influencing biomechanics, preprogramming shape and response complexity through fabrication and inert substrate interactions, to produce a palette of HBCs. Morphological aggregation was designed to harness and scale-up the HBC palette into programmable humidity responsive aperture openings. This culminated in pilot performance testing of a humidity-responsive ventilation panel fabricated with aggregated *Bacillus* HBCs as a bench-top prototype and suggests potential for this novel biotechnology to be further developed.

1. Introduction

Modern architectural environments are often static, unresponsive, and subsequently very energy demanding in their efforts to remain habitable within constantly changing environments. Nature, conversely, is a master of adaptation where seemingly static natural structures respond intrinsically, and frequently passively, as actuators to compensate for diurnal rhythms of temperature, humidity, and light (Park and Chen 2020). Aspiring to exploit these natural passive actuators has the potential to minimise energy consumption within the ever-evolving field of '*Responsive Architecture*' (Zhan *et al* 2023) and provides the impetus for this study investigating the development of spore-based hygromorphs as passive actuators. This paper reports on experimental work at multiple scales designed to understand the factors involved in programming this new class of spore-based hygromorphic materials including biological factors, physical factors, and morphological factors.

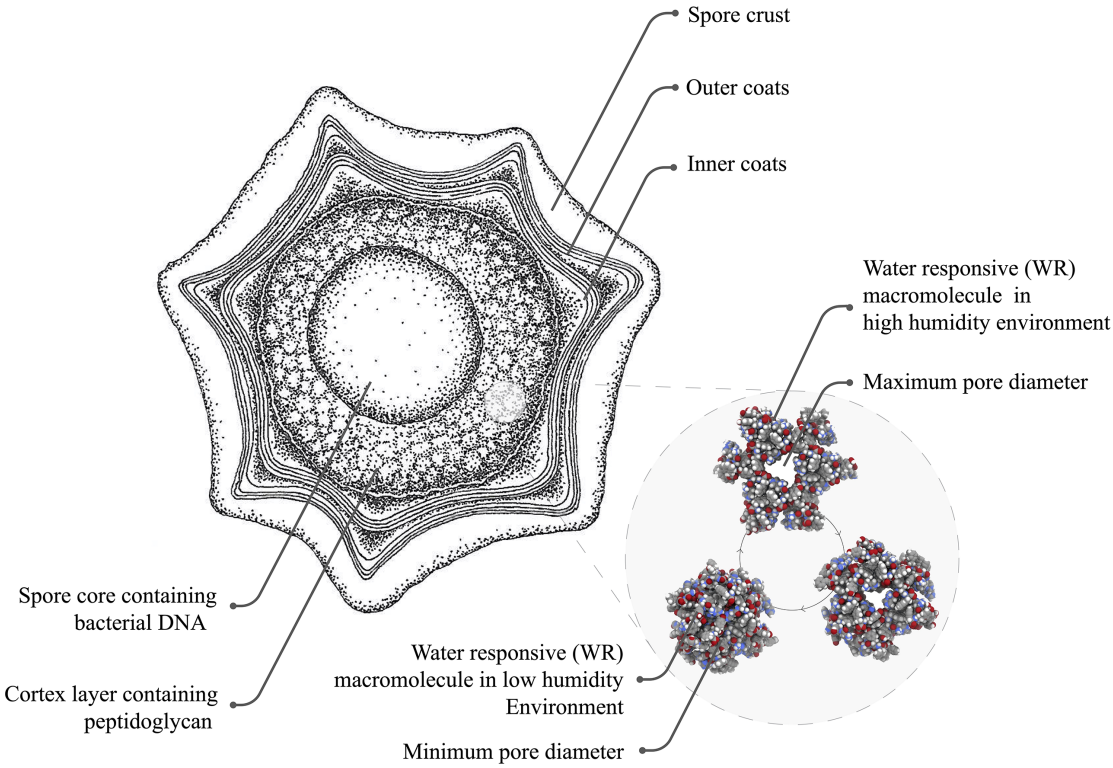

Figure 1. *Bacillus subtilis* spore structure and proposed functional morphology. The humidity driven change in nanopore diameter of water resposnice (WR) tripeptide crystal proxies, (adapted in part from Piotrowska *et al* (2021), with permission from Springer Nature) shows similarities to peptidoglycan's nanoscale amphiphilic pores which impacts reversable hydratin and molecular expansion (Wang *et al* 2022).

Hygromorphs are moisture sensitive materials which change their morphology, passively, in response to changes in environmental humidity (Park and Chen 2020) and can be said to 'compute' as their configuration (output) changes in response to external inputs (relative humidity (RH) or wetting) (Ramirez-Figueroa *et al* 2016). The use of hygromorphs within '*Responsive Architecture*' is not a new concept, and the natural adaptive properties of wood has been used for centuries to control ventilation in response to RH without any energy input (Larsen and Marstein 2000, Reichert *et al* 2014, El-Dabaa and Abdelmohsen 2023). However, deflections are predetermined by the growth dependant cellular morphology of the tree which limits actuation programmability. While this has been largely overcome by using complex wooden laminates (e.g. Wood *et al* 2018), they are vulnerable to environmental degradation (Holstov *et al* 2015).

The field has progressed to state-of-art, plant fibre–polymer composite, 3D-printed structures (Kam *et al* 2022). These complex hygromorphic composite materials use fused filament fabrication to create dynamic structures capable of complex kinematic deformations in response to environmental stimuli (for state-of-the-art review see de Kergariou *et al* 2023). The first habitable building to include such materials, The 'LivMatS' Biomimetics Shell, incorporates a hygromorphic solar shading system, 'Energy Gate' and *in-situ* performance testing of the system is currently underway.

An alternative and novel exploitation of hygromorphs in architecture has been digitally piloted to assess their 'moisture sink' and evaporative cooling properties. A roof aperture lined with a cooling hygromorphic hydrogel membrane was simulated to predict its impact on improving ventilation performance in hot-arid climates (Aviv *et al* 2020).

This paper investigates utilisation of a new class of hygromorphic material for architecture based on *Bacillus subtilis* spores. *B. subtilis* is a rod-shaped, soil bacteria (metabolically active, vegetative cells) capable of producing robust metabolically inactive bacterial spores when exposed to hostile conditions. These spores have a complex layered morphology (figure 1) designed to desiccate the DNA within the central core to inhibit metabolism and preserve it for, potentially, thousands of years (Nicholson *et al* 2000, Henriques and Moran Jr 2007). This desiccation is achieved by the hygromorphic properties of the cortex layer, surrounding the core, which contains a macromolecule called peptidoglycan (Driks 2003). This complex macromolecule is the 'engine' behind the spores' hygromorphic response. It is not only responsible for preserving the DNA, but also likely the physical expansion and contraction the spore undergoes in response to a change in humidity (Piotrowska *et al* 2021). This harnesses a

mechanism of water binding which changes the 3D structure of water responsive (WR) macromolecules by opening and closing nanoscale amphiphilic pores, and releasing energy through the hydration cycle (Piotrowska *et al* 2021, Wang *et al* 2022). *B. subtilis* spores are capable of expanding by 12% in response to changes in humidity (Chen and Mahadevan 2013), which is likely resultant of this macromolecular 3D shape change together with the impact of spore coat layers on the movement of water. The complex tissue architecture which facilitates this process is influenced by the conditions the bacteria are exposed to during culture and sporulation. Consequently, it was postulated that the bacteria could be preprogrammed to give a greater hygromorphic response by manipulating this environment through controlling culture conditions.

Inclusion of *B. subtilis* bacterial spores within a bilayer produces a spore-based hygromorph or HBC capable of harnessing the bacterial spores' humidity responsive capabilities from a simple expansion and contraction into a predictable deflection. These bacterial spores offer several promising characteristics and benefits over wood or cellulose-based hygromorphs when considered as part of a responsive architectural application, including rapid response time, sensitivity to small changes in ambient humidity, resilience in an architectural environment and, if effectively harnessed, an extremely high actuation energy density (Park and Chen 2020). These spores have been successfully employed within a bilayer to build the first nano-engines to harvest evaporative energy (Chen *et al* 2014), and fabricated as humidity responsive textiles to produce wearables with ventilation flaps (Wang *et al* 2017). However, in the architectural field this spore-based hygromorphic material is relatively unexplored. Their potential for architectural application has been introduced previously (Ramirez-Figueroa *et al* 2016, Birch *et al* 2021) and initial programmability studies based on spore concentration has also been reported (Birch *et al* 2021). Here this preliminary work has been extended by investigating biological, physical, and morphological factors determining the materials' programmability. These findings were used to develop a benchtop prototype fabricated to include aggregated *B. subtilis* spore hygromorph bio-composites (HBCs). The humidity-responsive performance was tested and presented in this paper along with the potential for further development of this novel biotechnology.

2. Methods

2.1. Biological factors optimisation

To optimise growth conditions, *B. subtilis* strain 168 vegetative cells were grown, subsequently sporulated, and evaluated for final spore concentration, culture volume yield, and magnitude of deflection (as a measure of hygromorphic response) under a range of protocols. This included the impact of nutrient availability, aeration, Fe^{2+} availability, washing protocol and culture volume.

This led to the development of an optimised sporulation protocol. During a two-phase growth period, the vegetative cells were provided with abundant aeration and essential cations such as Fe^{2+} to support development of peptidoglycan and the complex chemical morphology of some specific coat proteins responsible for the mechanics of the spore's adhesion properties. After incubating in 10 ml Luria-Bertani (LB) medium (Sigma) at 37 °C in a shaking incubator at 200 rpm for 12 h, the culture reached early exponential growth (achieving an OD_{600nm} of 0.8 (Abhyankar *et al* 2011)), and was transferred to a larger volume (90 ml to make 100 ml culture) of LB culture medium with the same chemical composition. This secondary, 2–3 h incubation in the larger volume culture promoted a high yield of actively dividing bacteria without limiting cell division through nutrient limitation. The cultures were then washed with phosphate buffered saline (Sigma) to remove any remaining LB media through a centrifuge–discard–resuspend method. A third and final 800 ml culture was used to initiate sporulation through restriction of available nutrients within the specialist sporulation media (Difco sporulation media adjusted to a pH of 7.6) (Sigma). The sporulation process was monitored through Schaeffer–Fulton staining (Sigma) every day from day 3 by heat fixing a 1 ml sample to a microscope slide and dyeing with Malachite green (step one of Shaeffer–Fulton staining method) slowly over 15 min. The slide was then counter stained with saffron (step two of Shaeffer–Fulton staining method) and light microscopy was utilised to visualise this sporulation progress.

2.2. Physical factors

Production of bacterial spore HBCs provides opportunities to 'design in' control parameters to programme computational responses from the material beyond that achieved by programming the biological factors through optimising sporulation protocols. A previous study (Birch *et al* 2021) demonstrated programmability of deflection magnitude through increasing and decreasing the spore concentration used to produce the HBCs. To build from this within this paper the potential for programming through physical factors was investigated:

- Inert substrate: the impact of substrate type on magnitude and repeatability of deflection.
- Material thickness: the potential to preprogramme a deflection magnitude through substrate material thickness.

Table 1. Design factors explored.

Design factor	Variations explored
Material	Latex Silicone TPU
Material thickness	0.2 mm 0.35 mm 0.4 mm 0.5 mm
Material etching	None 0.2 mm intervals Localised

- Etching: the potential to control deflection form (etching direction and effect of localised etching investigated).

These physical factors were investigated by producing a material pallet of bacterial spore HBCs, each exploring a different design factor (table 1).

All substrates were cut with a standard laser cutter (with a cutting resolution of 0.2 mm). The exposed edges were dabbed with blotting paper to remove any excess molten material and cleaned thoroughly with ethanol (Sigma). Once clean, the effect of adding a biological glue to aid spore adhesion was investigated by treating some samples with 8 μl cm^{-2} of poly-L-lysine (Sigma) and drying for 3 min until the glue layer became tacky before the spore layer was applied. The impact of substrate etching (transverse, longitudinal and diagonal etch lines, and the effect of localised etching) on deflection programming was also incorporated into the HBC material pallet.

The introduction of etching has enabled us to investigate the potential to produce fold versus bend deflections. In the first study two identical hexagonal stars were laser cut from 0.4 mm latex, with one being fabricated with 16.6 μl spore solution spread uniformly across each of the six star triangles (1 cm isosceles) and the second hexagonal star being laser etched across each of six fold regions. The same spore volume was applied discretely along the etching line. In the second study a simple 2D cube net (square face, 2 $\times$ 2 cm) was laser cut from 0.4 mm latex, etched at each of the 'fold' vertices and 16.6 μl of spore solution applied discretely along the etching lines.

The effect of spore concentration on deflection was also included in the range of samples prepared for the material pallet (volumes ranged from 4.2 to 33.2 μl cm^{-2}). Samples were placed on a rocking shaker to ensure no pooling occurred during the drying process, after which, samples were incubated at 99% RH for 24 h to equilibrate the deflection response programmed into the material through its fabrication protocol (figure 2).

A new methodology for measuring deflection angle was also developed to allow post analysis of sample photographs. A small, single sample rig was developed to ensure exact placement of each sample to produce photographs, all with the same set reference point (taken using Canon EOS 1300D, with electro focus short (EFS) 18–55 mm and Canon compact macro lens electro focus (EF) 50 mm, 1:2.5 with image stabiliser control camera, set on a fixed tripod) for analysis. Using Adobe Illustrator these results photographs were post analysed and used to map the deflection arc of each side of the HBC sample by producing a circle. These circles' sizes were determined by overlaying onto the photograph, following the deflection arc of the HBC as closely as possible. The centre point of these circles identified the centre of the defection arc. The start point of the deflection was defined as the edge of the spore deposition area on the latex and the end point of deflection was defined as the point of the end of the actuator. The angle between these two lines produced a segment from which the deflection angle could be measured. This geometric approach to data collection provided an accurate deflection angle (taken from segment produced) for each side of the HBCs. The relationship between substrate thickness and deflection angle achieved at 42% RH was presented graphically using Excel and analysed statistically using linear regression.

2.3. Morphological factors

Exploration of the physical factors produced a material palette which provided the data to select fabrication combinations required to produce HBCs which would deform at a given rate and a given magnitude across a given range of environmental RH. This provided the platform for the next stage of development which focused on programming morphological factors. Morphology here conveys a meaning of '*functional shape change*', with deflection (shape change) of the HBCs creating an opening aperture which functions to increase the exchange area for air flow as a passively responsive ventilation panel. A 3D printed (Ender 3 Pro) modular casement system was designed within Rhino® to allow clamping of six HBC actuators within one module. This modular system used a stud and socket connection design, producing a 'Lego' style aggregation to harness and scale the HBC palette into programmable humidity responsive apertures. This framework could house a range of different samples for testing (figure 3).

To measure the open area of each aperture they were incubated at 99% RH for 30 min before being removed from the humidity chamber and exposed to room humidity (42%) and filmed (using Canon EOS 1300D, with EFS 18–55 mm and Canon compact macro lens EF 50 mm, 1:2.5 with image stabiliser control camera on a tripod) for a further 30 min. Stills

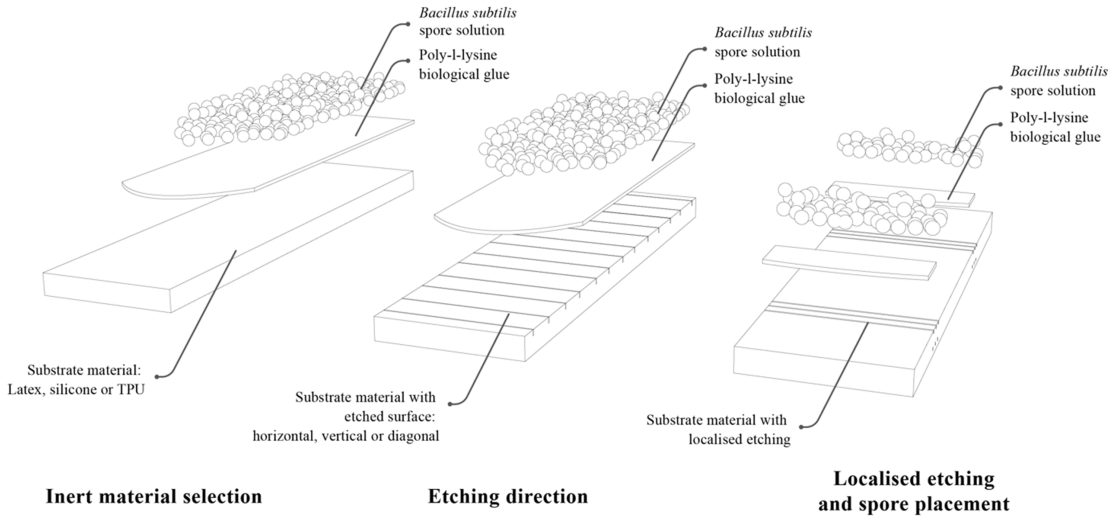

Figure 2. Hygromorph bio-composite bilayer fabrication.

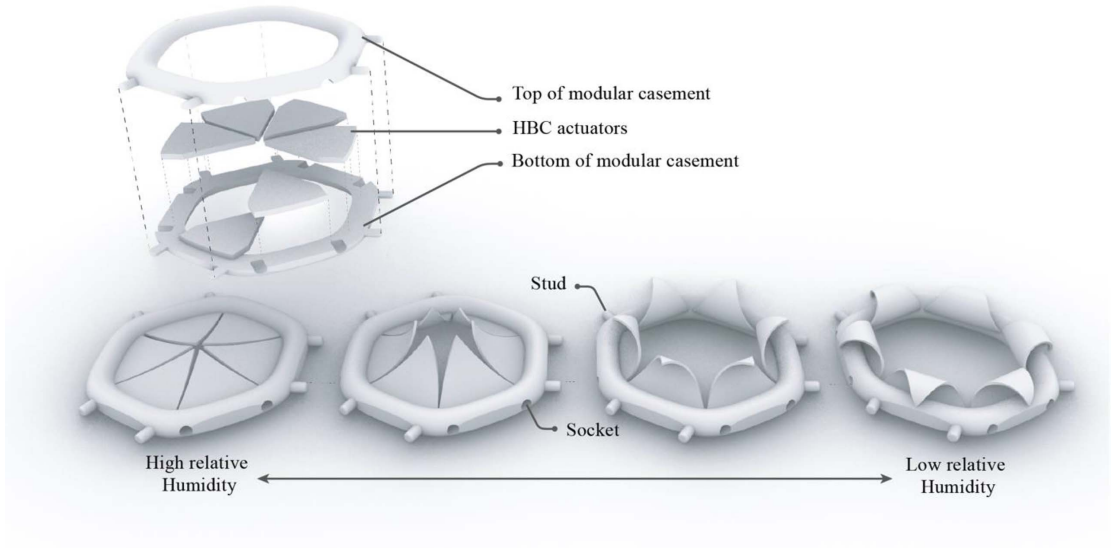

Figure 3. 3D printed modular casement system designed to house six HBC actuators developed within Rhino®. Once clamped within the casement system, multiple apertures created from the HBC material pallet may be connected to produce a unique configuration of responses to changing environmental humidity.

were taken every 3 min from this recording and analysed. The 'Image Trace' tool within Adobe Illustrator was used to isolate the open area within each aperture, then using the 'Dynamic Measure' plug-in a quantifiable value of aperture area at a range of RH could be obtained. This method was repeated three times to provide a triplicate data set. This provided understanding of each apertures' open area range across its environmental humidity range.

Performance testing of these apertures focused on assessing the HBC's potential for application within the built environment as a passive trickle ventilation membrane. To explore this, a new casement system was developed to house one type of HBC, producing a benchtop prototype panel. These benchtop prototype panels consisted of a 3D printed polylactic acid (PLA) honeycomb lattice casement system, hosting 27 discrete apertures, formed from six actuators of 0.4 mm latex and 8.3 μl cm^{-2} of *B. subtilis* spore solution per aperture. This HBC type was selected from the materials pallet due to having the greatest range in open area and speed of active shape change.

This panel of 27 apertures was subjected to the same methodology as with the individual aperture investigations, however, to capture the aggregated response all of apertures open area range were recorded and combined to give a maximum and minimum open area for the panel as a whole system.

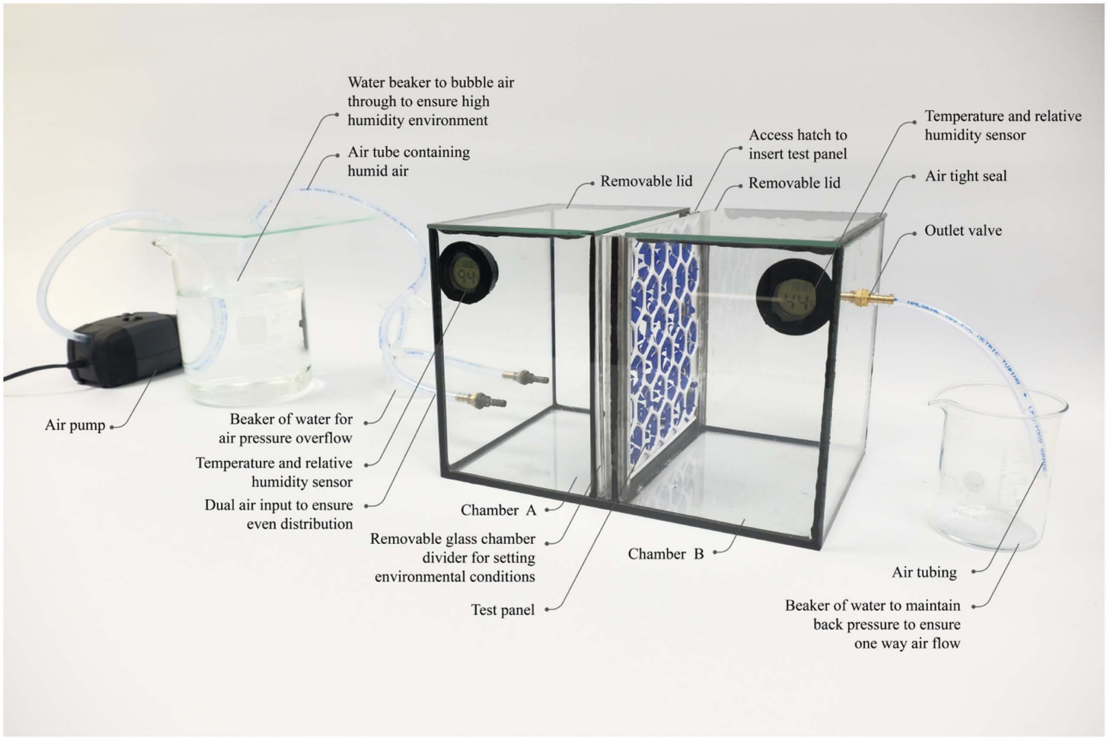

Figure 4. Experimental humidity test chamber.

The humidity test chamber (figure 4) was designed which facilitated the repeatable creation of artificial environments to explore the HBC benchtop prototype behaviour and performance at regulating airflow between two contrasting RH environments. This was achieved by subdividing a glass container to provide two adjacent controlled humidity environments. They were separated by the test panel which was airtight excepting airflow through the open aperture areas. The temperature and humidity of each side could be monitored using separate surface mounted humidity monitors (EEEkit digital hygrometer). The humidity of the air entering the first chamber was controlled through use of an air flow pump connected to an air stone within a water bath providing a >99% RH airstream. The second adjacent chamber contained dry air which was achieved by using anhydrous calcium sulphate as a desiccant (Drierite) to provide a contrasting minimal RH environment.

3. Results and discussion

The current study investigates bacterial spore based hygromorphic materials spanning across many different fields including microbiology, materials science, design, fabrication and architectural technology, and journeys from the ultracellular through to bench-top prototype (figure 5), adding the challenges of scale and dimension to the approach. Reflecting this, a phased, multidisciplinary methodology, building refinement and programmability at each scale of development of this emerging material is presented. It explores the potential computational parameters, both biological and design which can push this emerging biomaterial toward responsive architecture from the cellular level to a bench-top prototype.

3.1. Biological factors

Firstly, the biological factors at the cellular and ultracellular level were explored through the optimisation of growth and sporulation conditions to biologically preprogramme optimum spore hygromorphic response and yield. Previous growth and sporulation methodologies to produce *B. subtilis* spores for use as hygromorphs (Chen and Mahadevan 2013, Yao *et al* 2015, Ramirez-Figueroa *et al* 2016) have utilised a 'standard' LB culture methodology which does not target optimisation of hygromorphic capabilities of the spores. Within this paper, optimised protocol was developed and hygromorphic capabilities of enhanced spores was investigated (figure 6). The results show that the optimisation of the *B. subtilis* vegetative cell growth and sporulation protocols with 3× the hygromorphic response (70.7° compared to 220° for the optimised protocol) observed through HBC deflection (for comparable HBC fabrication) (figure 7) and produced a 40 fold increase in spore yield per batch (8 ml) in comparison to spores produced in the previous studies (0.2 ml) using a standard LB-based media culture and Difco sporulation media (DSM) sporulation protocol (Birch *et al* 2021). The authors are not aware of other studies

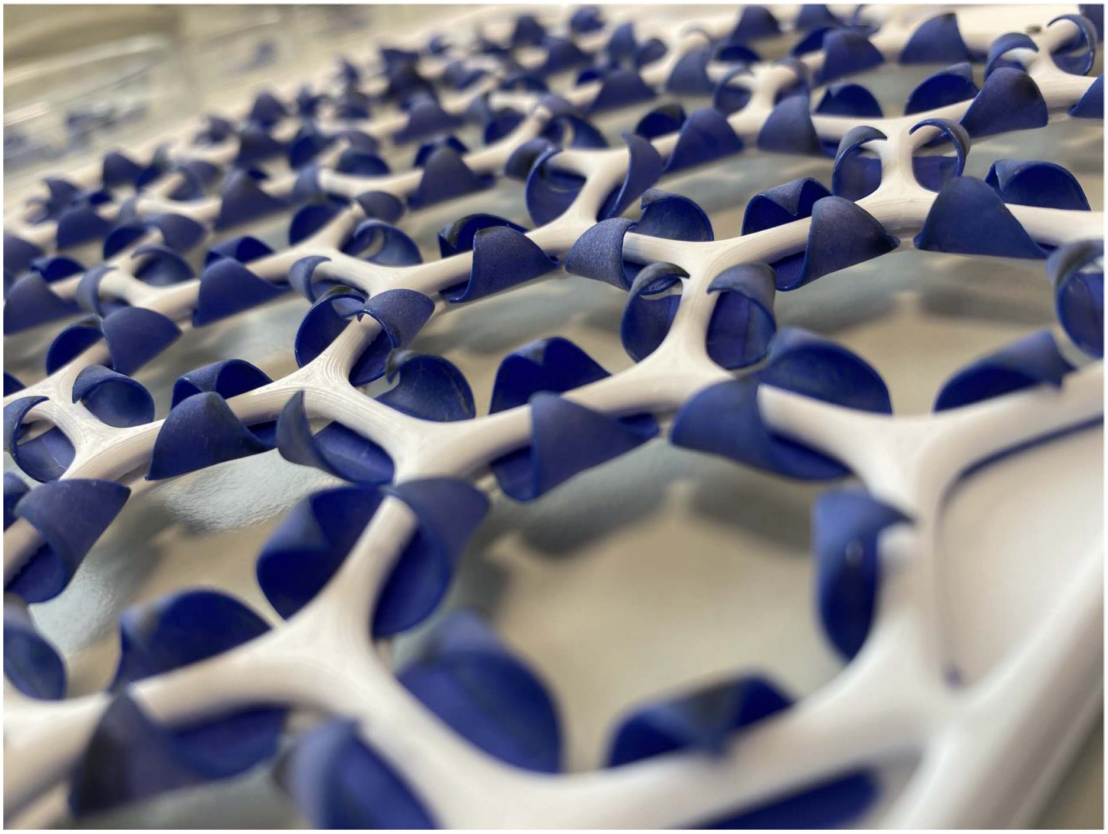

Figure 5. Benchtop prototype of a humidity-responsive ventilation panel (400 cm^2) with six aggregated *Bacillus* HBCs housed to facilitate humidity responsive changes in the open cross-sectional area of the aperture.

where magnitude of spore specific hygromorphic response has been evaluated. This increase in hygromorphic response is likely to reflect an increase in peptidoglycan content of the cortex layer of the spores in optimised conditions as proxy studies have suggested the hygromorphic expansion force is generated by binding of molecular water in the porous structure of this macromolecule (Piotrowska *et al* 2021). The optimised sporulation protocol provided an increase in aeration and nutrient supply which also resulted in an increase in spore yield. This reflects similar findings by Elisashvili *et al*, which were attributed to enhanced reproduction rate of vegetative bacteria with higher nutrient availability, and a higher sporulation % in aerobic conditions (2019).

3.2. Physical factors

After the optimisation of biological elements, material explorations were followed, to investigate how physical factors influence biomechanics, to preprogramme shape changes and response complexity through fabrication and inert substrate interactions, and to produce a palette of HBCs.

3.2.1. Substrate material selection

Three flexible materials were investigated as potential substrates to produce HBC actuators: latex, silicone, and thermoplastic polyurethane (TPU). Latex has previously been used with poly-L-lysine to successfully to produce *Bacillus* HBCs (Birch *et al* 2021). This study has further demonstrated that without poly-L-lysine there is delamination between the spore layer and latex after ten deflection cycles (figures 8(d) and (e)). However, latex deteriorates when exposed to UV light and has limited potential for 3D fabrication so explorations into alternatives were explored. Silicon is a synthetic alternative to latex, and offers options for more complex fabrication methods, while TPU has the advantage of being readily fabricated using advanced 3D-printing technologies. Both alternatives were investigated with the use of poly-L-lysine. The results demonstrated that HBCs fabricated with TPU showed no deflection (figure 8(c)), while those fabricated with silicon showed a deflection of a similar magnitude to that of latex initially (figure 8(a)), but the bio-composite was not as resilient and failed after the third hydration–dehydration cycle (figure 8(b)). Recent work has suggested some success with a silicone adhesive which may offer opportunities to work with silicone in future studies (Wang *et al* 2022).

3.2.2. Substrate thickness

Building upon the spore concentration findings from the previous work (Birch *et al* 2021), this study has determined that increasing the thickness of latex used

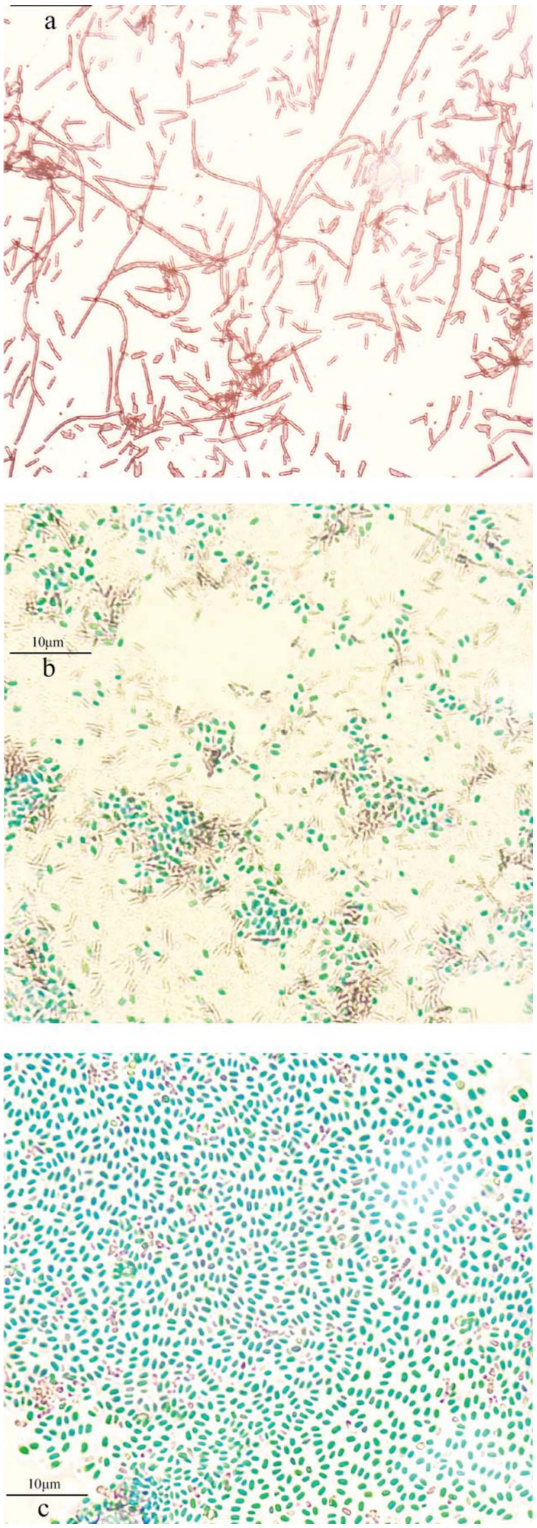

Figure 6. Visualisation of *B. subtilis* sporulation process, using Shaeffer–Fulton staining method. Spore percentage increases during nutrient limited incubation in DSM media at day 1 (a), day 3 (b) and day 5 (c). Vegetative (metabolically active) cells stained in red, and spores stained in blue–green.

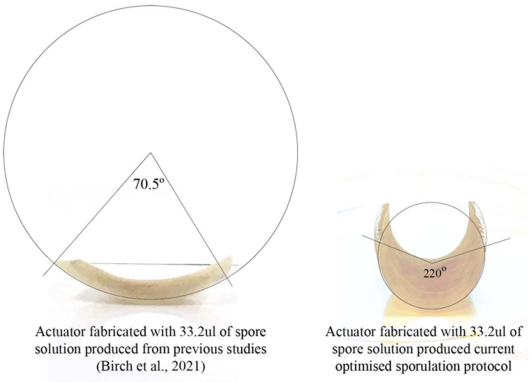

Figure 7. Comparative hygromorphic deflection response for optimised sporulation protocol.

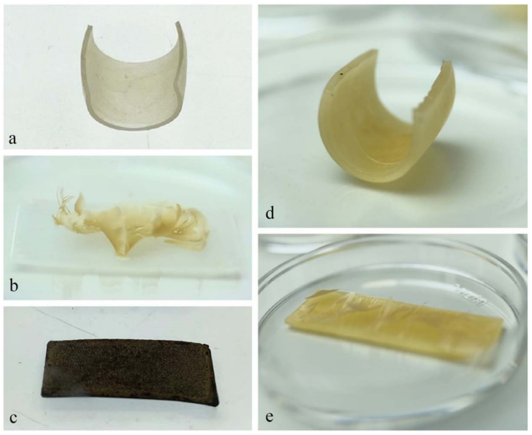

Figure 8. Explorations into suitability of substrates for production of HBCs (a) 0.5 mm silicone, initial deflection (b) 0.5 mm silicone, catastrophic delamination after three deflection cycles (c) 0.5 mm TPU, no deflection response (d) 0.5 mm latex with poly-L-lysine demonstrating large deflection (e) 0.5 mm latex without poly-L-lysine showing catastrophic delamination after ten deflection cycles.

to form a HBC decreases the deflection angle achieved (figure 9, represented graphically in figure 10) and through linear regression it has been demonstrated there is a direct correlation between the two variables with $R^2 = 0.9968$ and as such, a desired deflection angle may be preprogramed into the material at fabrication through this parameter. It also promotes the concept that the magnitude of deflection is programmed by the actuation stresses generated by the differential expansion of the two layers of the bilayer, since, an earlier study (Birch *et al* 2021) demonstrated that a higher concentration of spores in the bilayer fabrication resulted in an increase in magnitude of deformation.

3.2.3. Material etching

Etching investigations yielded promising results with repeatable deflection magnitude and direction achieved. Etching the top surface of the latex prior to coating with biological glue and *B. subtilis* spore solution resulted in the sample deflecting perpendicularly to the etch direction and consequently a twist, curl and roll deflection may consistently be preprogramed during fabrication (figure 11).

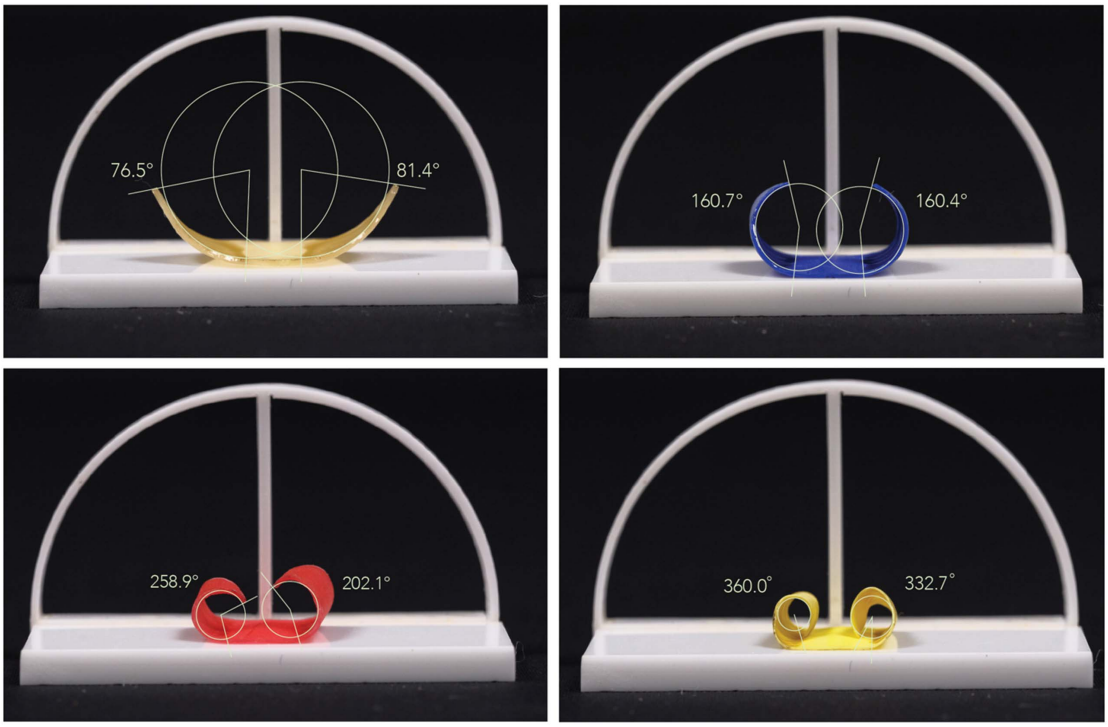

Figure 9. Hygromorph bio-composite samples formed from latex of a range of thicknesses (0.5 mm natural, 0.4 mm blue, 0.35 mm red, 0.2 mm yellow) showing deflection angles achieved at room humidity 42% RH.

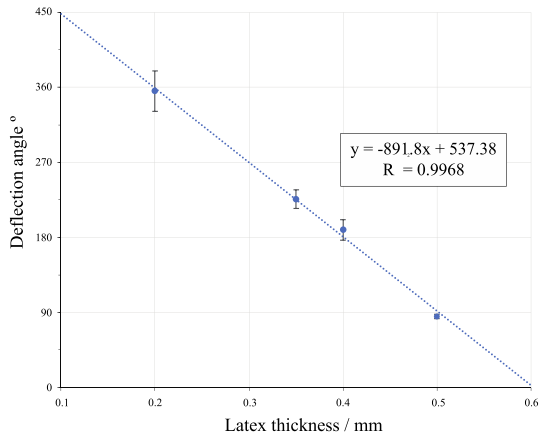

Figure 10. Negative linear relationship between maximum deflection angle for HBCs at 42% RH and substrate (latex) thickness. (12 replicates for each latex thickness/mm, data given as mean ± SEM).

This finding supports recent studies (Zuo *et al* 2023), where they were investigating directional deformation with hydrogel bilayers as soft actuators. They also adopted a laser etching methodology to create patterned surface markings, which resulted in similar twist, curl, and roll deflections. These findings together suggest laser etching reduces bending stiffness of the latex in one direction resulting in effective preferential bending, thereby programming the deformation. Localised etching and spore deposition resulted in a fold rather than curve deflection (figure 12).

This folding response was also demonstrated in the simple self-assembly folding pilot study. It was observed that a 3D open-topped cube could be successfully self-assembled from a 2D net at room humidity and returned to its two-dimensional form in a high humidity environment (figure 13) mimicking some of the much more refined hydrogel self-assembly demonstrations by Tibbits (2014). The extent of the fold angle was approximately 90°–100° and lower than that seen with the hexagon star study where folding reached a 180° angle. However, the folding of the faces of the self-assembly cube

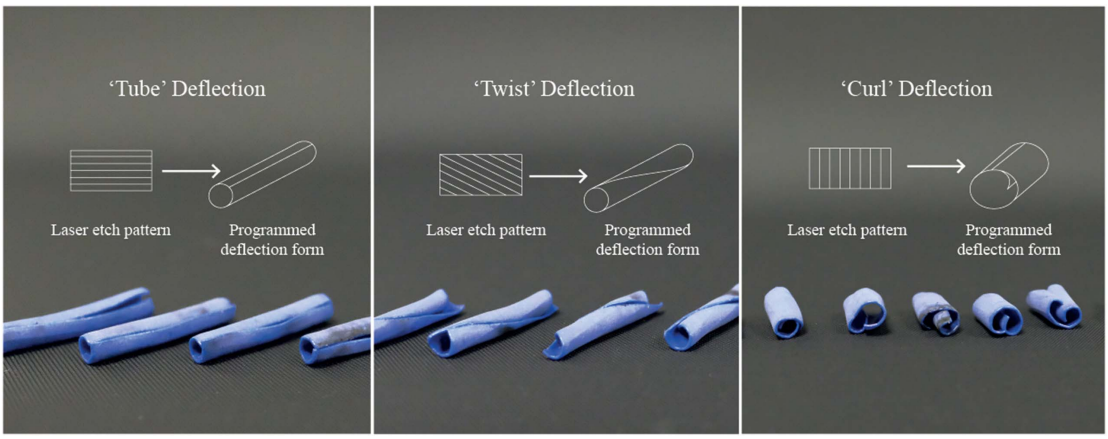

Figure 11. Pre-programmed responses observed in hygromorph bio composites with etching patterns at 42% RH. Left to right—tube deflection, twist deflection, curl deflection.

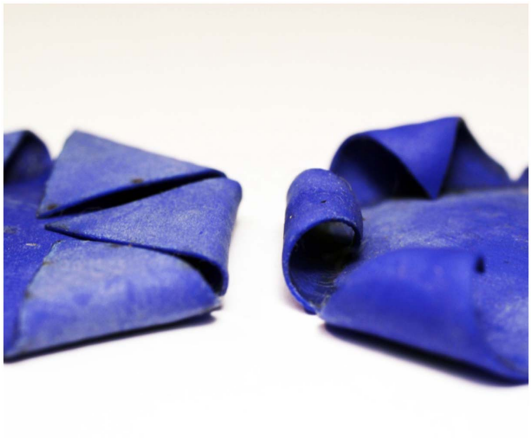

Figure 12. Comparison of fold and bend deflections.

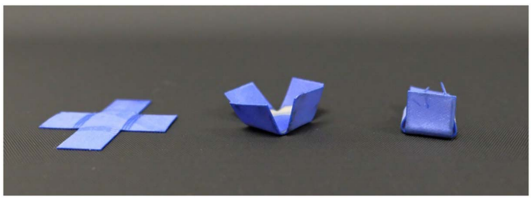

Figure 13. Localised etching and spore placement programmed a fold deformation which led to a self-assembly pilot of an open cube.

were restricted because they overlapped with other faces.

3.3. Morphology factors

Latex thickness showed great potential to preprogramme an environment specific response within the aggregated system. Aperture size increased at a given RH as latex thickness used to fabricate the HBC decreased. These panel apertures were monitored over a 30 min period ranging from 99% RH to 42% and reached their maximum response within a 15 min active zone (figure 14). This fast response time of these spore-based HBC prototypes appears faster than the highly developed wood-based 3D printed apertures which respond within 35 min (Tahouni *et al* 2023). It is likely the increase in response speed is due to a short diffusion distance into uniform microscopic spores.

Image trace methods provided accurate mapping and evaluation of aperture kinematics, providing a detailed understanding of range of motion and activation points. It was observed that an 85% increase in aperture open area can be achieved when transferred between 99% and 42% RH. When interpreting these results, there is notable consistency between the 27 replicate apertures that combine to form the prototype panel, and repeatability of kinematic response over three repeated hydration–dehydration cycles showed low variation as seen from the error bars in figure 15.

Initial performance testing of these benchtop prototypes within the experimental test chamber highlighted a dual action response of the HBC apertures which contribute to their impact on RH of

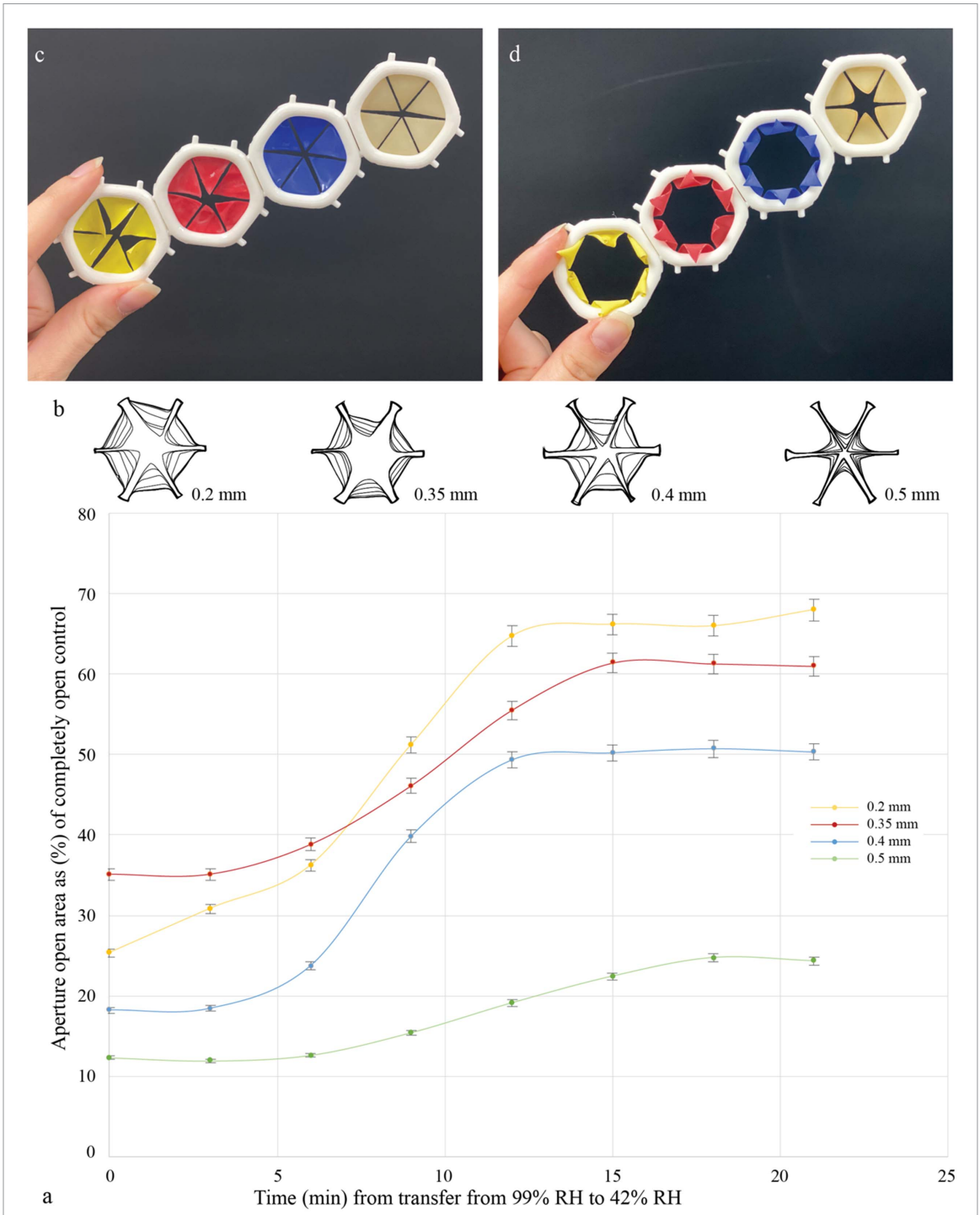

Figure 14. (a) Impact of latex thickness (0.5 mm—green/natural, 0.4 mm—blue, 0.35 mm—red, 0.2 mm—yellow) on open area of apertures as panels are transferred from 100% RH to 42% RH. ($n = 3$ for each latex thickness/mm, data given as mean ± SEM). (b) Image trace mapping aperture size at ten data points between 100% and 42% RH. (c) Apertures at 100% RH and (d) 42% RH.

the chambers. Firstly, as expected, the apertures close when exposed to high humidity environments, reducing the open area and rate of air diffusion across between the chambers. This maintained the humidity level in the chamber for longer within chamber B than the control because movement of humid air was reduced. Secondly, this study highlighted the bacterial spores' capabilities as a humidity sink. It was observed that as humid air passed through the HBC apertures, moisture was removed from the air. While spore water content was not measured directly, it is likely this moisture was retained by the spores. A humidity sink response was proposed with a simulated model using hydrogel (Aviv *et al* 2020) and this could suggest *B. subtilis* spores have a similar humidity sink capacity and are able to reduce RH of a high

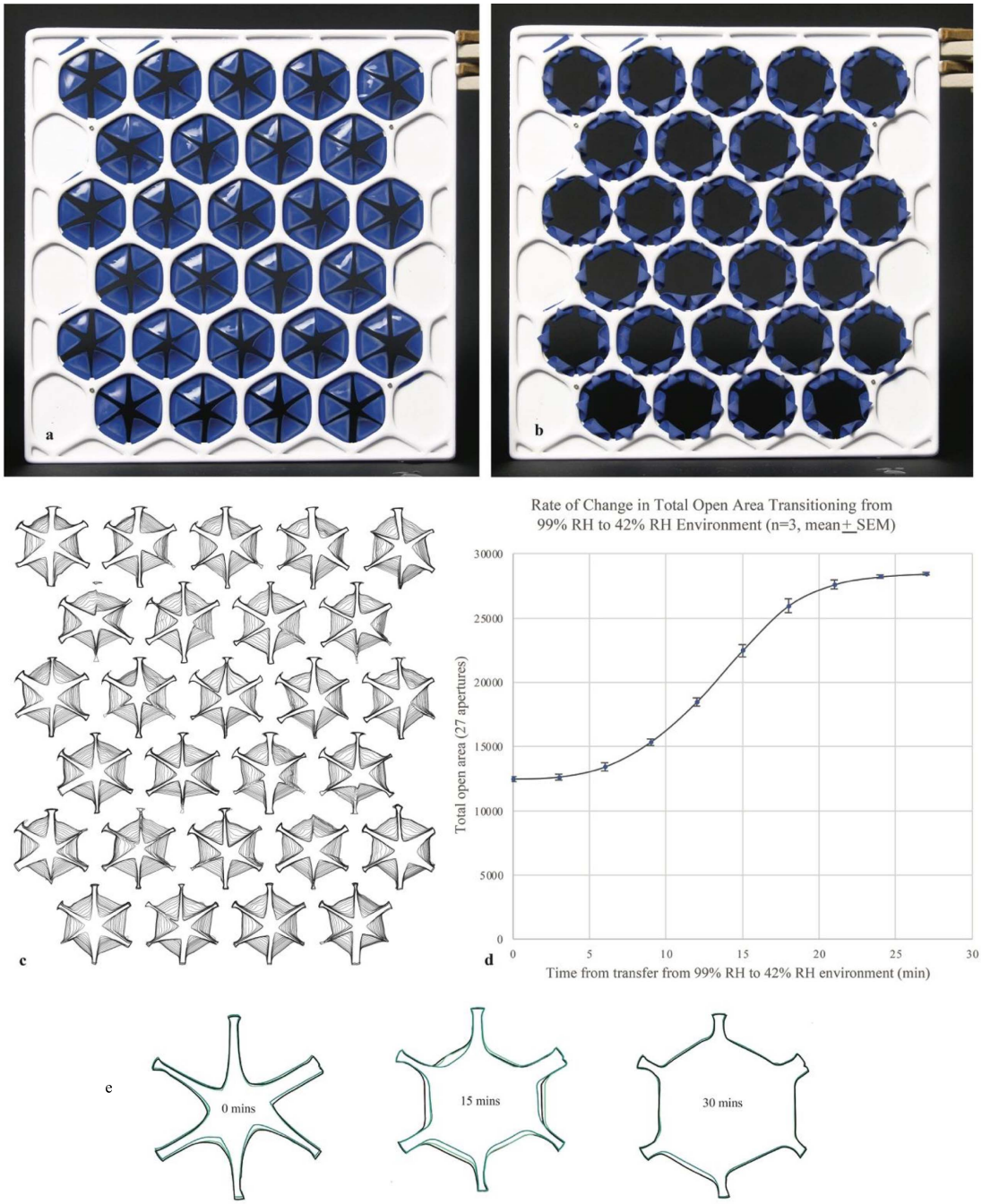

Figure 15. Prototype hygromorph bio composite panel hosting apertures made from 0.4 mm latex with 8.3 μl cm^{-2} of spore solution. (a) Panel exposed to 99% RH (b) panel exposed to 42% RH. (c) Image trace mapping aperture size at ten data points between 99% and 42% RH. (d) Time course of change in aperture open area (combined from the 27 individual apertures per panel) following panel transfer from 99% to 42% RH ($n = 3$ mean $\pm$ SEM). (e) Example triplicate open area image traces for one aperture at 0 min, 15 min and 30 min following transfer from 99% to 42% RH.

humidity environment. Conversely, this stored moisture could be re-released into the environment at low RH increasing the humidity. The concept of a hygromorphic material acting as a humidity sink and demonstrating the impact of material–environment-coupling on humidity gradients has also been demonstrated (Treml *et al* 2018).

4. Conclusion

These results demonstrate that modification of biological factors, by optimising the *B. subtilis* spore culture protocol, can triple the hygromorphic response of spores and increase the yield by a factor of 40 when compared to previous protocols (Birch *et al* 2021).

This optimised protocol used basic lab equipment, was efficient, and promises an accessible route to generate the supply of hygromorphic spores necessary to develop this emerging biomaterial. Explorations of physical control factors allowed preprogramming of deflection magnitude and direction, producing highly programmable HBCs. There was a negative correlation between latex thickness and deflection angles achieved, and laser etching the upper surface of the latex predetermined deflection direction. Furthermore, direct spore deposition on etched zones created a fold, rather than bend deflection, which led to successful pilot investigations into origami-based self-assembly. However, investigations of alternative substrate materials and adherence demonstrated that neither TPU nor silicone are currently suitable, although recent advances in silicone adhesive technology may open options for this more readily 3D fabricated material in the future. Utilising material thickness and etching, individually and in combination, allowed the HBCs to be preprogramed, and the actuators were then combined to enable them to open and close apertures within a modular casement. The HBCs produced repeatable and predictable 'open' and 'closed' morphology when exposed to changing RH. Factors affecting this morphology were investigated and demonstrated a highly consistent, negative correlation between latex thickness and open aperture area. A preliminary investigation was undertaken to performance test a humidity-responsive ventilation panel fabricated with aggregated *B. subtilis* HBCs as a bench-top prototype. It showed promising results, and an ability as a semi-permeable breathable membrane, to maintain a passively responsive interface between two contrasting RH environments. Furthermore, the benchtop prototypes capacity to moderate humidity was enhanced by its apparent additional action as a humidity sink.

Further explorations into developing these HBC prototype panels to refine performance capabilities and improve material robustness could propel this emerging technology closer towards architectural application.

Data availability statement

All data that support the findings of this study are included within the article (and any supplementary files).

Acknowledgments

This research was funded by Newcastle University's Research Investment Fund and E3 funding of laboratory facilities. The authors would like to thank staff and students at the Hub for Biotechnology in the Built Environment for their support.

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