
Article:
Dade-Robertson, M. orcid.org/0000-0001-7752-9605, Mitrani, H. orcid.org/0000-0001-9845-3291, Corral, J.R. et al. (4 more authors) (2018) Design and modelling of an engineered bacteria-based, pressure-sensitive soil. Bioinspiration & Biomimetics, 13 (4). 046004. ISSN 1748-3182

https://doi.org/10.1088/1748-3190/aabe15

Bioinspiration & Biomimetics

OPEN ACCESS

RECEIVED
19 November 2017

REVISED
28 March 2018

ACCEPTED FOR PUBLICATION
13 April 2018

PUBLISHED
24 May 2018

PAPER

Design and modelling of an engineered bacteria-based, pressure-sensitive soil

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Keywords: responsive materials, material based computation, biological sensors, synthetic biology, cementation, computational modelling

Abstract

In this paper, we describe the first steps in the design of a synthetic biological system based on the use of genetically modified bacteria to detect elevated pressures in soils and respond by cementing soil particles. Such a system might, for example, enable a self- constructed foundation to form in response to load using engineered bacteria which could be seeded and grown in the soils. This process would reduce the need for large-scale excavations and may be the basis for a new generation of self-assembling and responsive bio-based materials. A prototype computational model is presented which integrates experimental data from a pressure sensitive gene within *Escherichia coli* bacteria with geotechnical models of soil loading and pore water pressure. The results from the integrated model are visualised by mapping expected gene expression values onto the soil volume. We also use our experimental data to design a two component system where one type of bacteria acts as a sensor and signals to another material synthesis bacteria. The simulation demonstrates the potential of computational models which integrate multiple scales from macro stresses in soils to the expression of individual genes to inform new types of design process. The work also illustrates the combination of *in silico* (silicon based computing) computation with *in vivo* (in the living) computation.

1. Introduction

Biological systems are highly energy efficient, responsive and adaptable to change. For this reason, engineers often turn to biology for inspiration in the construction of human engineered systems. Modern computation and highly controllable fabrication processes such as 3D printing allow structures to be produced with spatially variable material properties, more similar to those found in nature, giving for example variable stiffness to a piece of furniture (Oxman 2010). This process is known as Material Based Design Computation (MBDC). Taking this MBDC idea further, responsive materials, which change their properties or geometry in response to external stimuli, offer potential for a more sustainable built environment which can adapt to changes in environmental conditions and requires less energy to construct, operate and maintain. Examples include passive architectural skins inspired by fir cones, which use hygromorphic materials to open and close in response to changes in ambient humidity (Holstov *et al* 2015, Reichert *et al* 2015). Some responsive materials go a step further and utilise living microorganisms which respond to environmental change. For example, self-healing concrete includes dormant bacteria and nutrients in the concrete mix which respond to cracking by precipitating calcium and closing the crack (Jonkers 2007).

We suggest that the next frontier in the development of adaptable materials is to utilise engineered biological systems so that computation, material synthesis and fabrication are combined into materials that are part living. This would go beyond bio-mimicry

or the use of naturally occurring organisms and materials to the development of biological systems which perform in ways which they do not do in nature. Our ultimate objective is to construct a responsive material which uses engineered bacteria as biological sensors to detect mechanical changes in their environment and to respond by synthesising new materials. An application of this would be, for example, to 'automatically' construct building foundations by bacteria within the soil matrix detecting changes in pressure and cementing the soil in response.

Using Synthetic Biology approaches to create bacteria capable of cementing soil in response to pressure is a promising scenario for two main reasons. Firstly, microbial activity is already important to many geotechnical processes, although this area has only been studied relatively recently (Mitchell and Santamarina 2005). Microbial activity is known to have a significant impact on the fertility of soils in the upper layers and bacteria are known to move freely through many types of soils through pore 'throats' which form a network of gaps between sediment grains. In unsaturated sediments bacteria tend to live at the surface in the area below the organic layer and activity decreases by one or two orders of magnitude with 2 m of depth. However, in saturated sediments the reduction in numbers of bacteria is more moderate (Mitchell and Santamarina 2005).

Secondly, bacteria have already been widely shown to cause the binding together of soil particles. Some bacteria can become attached to particles and form biofilm of single species or multi-species bacteria colonies. This build-up of biofilms can lead to greater adhesion of sediment particles. Bacteria are also responsible for cementing soils with known species such as *Sporosarcina pasteurii* and *Bacillus megaterium* producing ammonia which, in turn, causes calcium carbonate to precipitate (Decho 2010). This has been studied extensively as a promising method of ground improvement and microbial induced calcium precipitation (MICP) has been shown to produce significant enhancement in the mechanical properties of soils, both at small scale and in field tests (Dejong *et al* 2006, Whiffin *et al* 2007, van Wijngaarden *et al* 2011). This work shows the importance of factors such as bacterial concentration, pH, reactant concentration, temperature, and nutrient availability on the success of MICP, which highlights the complexity of biological cementation methods. However, these bio-cements are not responsive to their environment. Using these methods, cementation occurs wherever the bacterial solution is injected, not in response to an external input, such as pressure.

This paper presents the initial steps towards this application domain in the form of a design process which integrates gene expression data from a new engineered pressure-responsive bacterium into a computer model of mechanical soil behaviour. Using such a model we can potentially design the macro behaviour of soils by engineering the behaviour of microorganisms within the soil (micro scale) by altering their genes at the nano scale. The model considers a scenario where a raft applying a uniform load over its area is placed on a homogeneous, saturated volume of soil. The soil volume is saturated with water containing the engineered pressure sensitive bacterium. Ordinarily, when the soil is loaded, the water would be pushed out of the pores and the soil would consolidate, causing local settling of the ground surface and foundation and some increase of soil strength. However, in the proposed system, the bacteria respond to elevated pore water pressures induced by the load on the soil. In the model presented the response to pressure is demonstrated by raised levels of a reporter enzyme, green fluorescent protein (GFP), which we use to measure the response of our gene of interest. However, if a synthetic biological system was built where pressure sensitivity was connected to a system of material synthesis, these areas of increased GFP would represent regions of the soil model to be bio-cemented. This bio-cementation would bond the soil grains together, prevent settlement and increase the soil strength beyond any strengthening which consolidation would cause. The limitations of this model and next steps in the development of such a system are also discussed.

2. Background

2.1. Biological aspects

While there have been studies of the genetic response of bacteria to elevated pressure there have not been attempts to engineer a pressure sensing strain and there is little data on the response of bacteria to low levels of elevated pressure (up to 1 MPa). The first step towards creating such a pressure sensor is to identify and characterise genes which show a genetic response to elevated pressures. This can be done using a technique called RNA-Seq to characterise the response of the entire genome to pressure changes and search for potential pressure sensitive genes. We then use techniques from molecular biology to build a gene circuit and characterise the sensitivity of the genetic response.

2.1.1. Background to Synthetic Biology

The expression of many genes is regulated depending on whether the cell needs their products at a given time. This process of regulation, it is proposed, can be harnessed by building gene circuits. Regions of deoxyribonucleic acid (DNA) can be constructed using 'parts' and assembled into 'devices' represented by symbols (equivalent to an electrical circuit diagram) using SBOL (Synthetic Biology Modelling Language) visual and illustrated in figure 1. These parts can, for example, encode for proteins or interact with other molecules in the cell inhibiting or promoting transcription. A device also contains other parts necessary for transcription and translation including a terminator which prevents further transcription and ribosome binding sites which initiate translation from

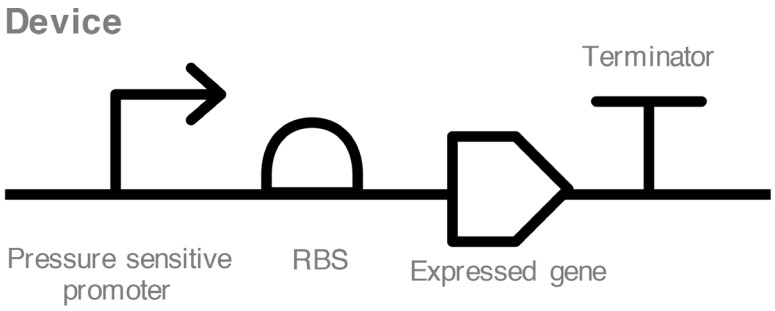

Figure 1. Diagram of a genetic 'device' consisting of a promoter which is the starting point of a gene transcription that ends at the terminator and generates a messenger ribonucleic acid (mRNA) and a ribosome binding site which recruits the ribosome which initiates the process of translation of the mRNA into a protein. The diagram is drawn using the graphical standard: SBOL Visual.

ribonucleic acid (RNA). By recombining promoters with genes that express different proteins we can create new genetic circuits. Expression profiles can be mapped for specific genes showing the genetic response (in terms of gene expression level) against a given input. In our case, we are interested in potentially pressure sensitive genes—i.e. genes that are regulated by changing pressures in the environment.

2.1.2. *Bacteria based pressure sensor*

The growth, survivability and adaptation of bacteria to pressure have been studied in terms of low pressures (below atmospheric pressure of 0.1 MPa), for example for *Bacillus subtilis* (Nicholson *et al* 2010, Waters *et al* 2014), moderate pressures (<100 MPa) and high pressures (>100 MPa), especially *Escherichia coli* (*E. coli*). In geotechnical contexts we are unlikely to see pressures of more than 10 MPa (in practice more likely to be between 0.1–1 MPa) and there is a lack of data for these low levels of elevated pressure. In a connected study, we identified and characterised pressure sensing genes in a lab strain of *E. coli*, focussing on gene responses at low elevated pressures (up to 1 MPa). The data and methods are fully described in Guyet *et al* (2018). Briefly, using RNA-Seq experiments, we identified 75 genes that displayed a significant change in expression under pressure treatment with a cut-off of fold change ⩾3. Among these, 69 genes were upregulated when the cells were exposed to 1 MPa up to 30-fold difference and 6 genes downregulated up to 5-fold, compared to untreated cells. Nearly half of the upregulated genes (30) were also found upregulated when *E. coli* cells were exposed to higher pressures (30 MPa, 50 MPa and 100 MPa). We then chose one gene candidate that showed a significant gene expression change (*azuC*) to create a pressure sensitive GFP reporter strain P_{azuC} *azuC-gfp*. We successfully monitored the pressure response of the *E. coli* AG1319 strain (P_{azuC} *azuC-gfp*) by measuring the GFP signal under pressures of 0 MPa, 0.2 MPa, 0.4 MPa, 0.6 MPa, 0.8 MPa and 1 MPa. The signal was measured in relative fluorescence units (RFUs) with increased fluorescence indicating an increase gene activity in response to pressure. The results are reproduced in table 1 and the normalised signal data were used in our computational model, as described in section 3.2.

Table 1. Engineered strain response to different pressures after 3 h exposure to elevated pressure.

	Engineered pressure sensitive strain[a] (RFU.OD_{600}^{-1})	
Pressure range (MPa)	Under pressure	No pressure (control)
1	4039.57	2841.80
0.8	3267.09	2621.15
0.6	3322.25	2573.26
0.4	3534.06	2896.22
0.2	3196.22	2846.09
0	2934.53	2892.84

[a] *E. coli* AG1319 strain (P_{azuC} *azuC-gfp*), expression of *gfp* was controlled by pressure sensitive promoter identified by RNA-Seq experiment.

2.2. Background to soil mechanics

In saturated, unloaded soils, where all the pore spaces are filled with water, a hydrostatic pressure distribution with depth exists. However, as the saturated soil is loaded, localised increases in pore pressure, known as excess pore pressures, can occur before water is able to flow out of the pores and pressure is equalized throughout the system. Pore pressure is therefore a function of:

- The magnitude of the applied vertical stress
- How quickly the pore water is able to drain, which depends on the permeability of the soil and the length of the drainage path (Powrie 2014).

This dissipation of excess pore pressures is accompanied by compression of the soil matrix. The restructuring process of a soil under loading is known as *consolidation* (Terzaghi 1925). Consolidation is most apparent in clay soils underneath building foundations and other structures, where water can only drain slowly, as the process happens almost immediately in more permeable soils such as sands. The equa-

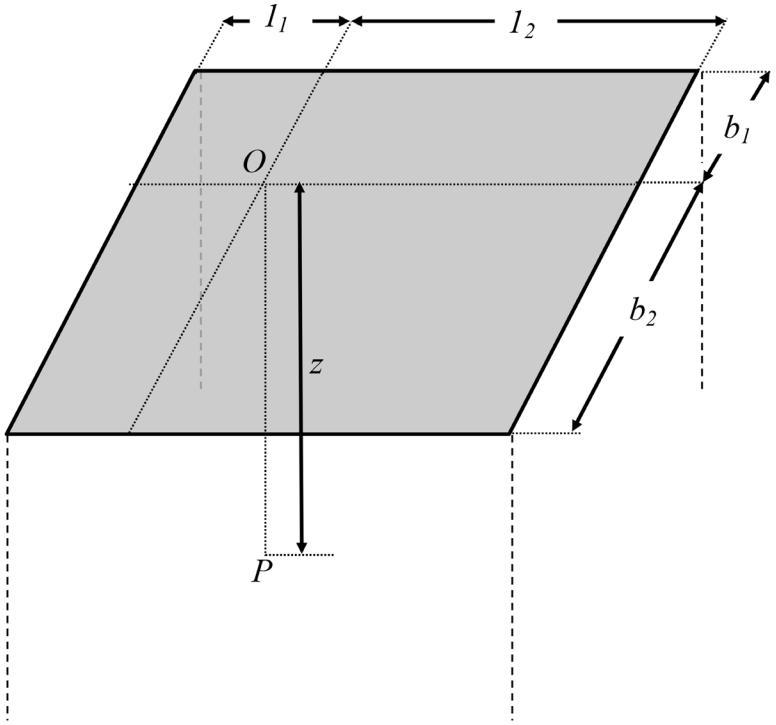

Figure 2. Graphical representation of a rectangular foundation for calculation based on Boussinesq's analysis (Fadum 1948) of vertical stress at a point below.

tions governing this process and used in the computer model are described in the following sections.

3. Methods

3.1. Development of the computational model

There are two main components to this model: the biological behaviour of the bacteria in response to pressure and the mechanical behaviour of the soil. The biological behaviour is based on gene expression data outlined above (obtained using the *in vivo* experiments described fully by Guyet *et al* (2018)) as well as using hypothetical data to explore different design scenarios. The mechanical behaviour of the soil, in this case consolidation behaviour, is represented by a static model of total vertical stress combined with a time-dependent model of pore pressure.

The program used to develop the model was Processing (v.3.0.1). This software is based on the Java language and is mainly used for visualisation. The code implements a type of finite element analysis where the area underneath the loaded foundation is split into voxels. This is done by building a 3D array consisting of a grid of points below the area of the foundation for a given depth. At each point in the grid, vertical stress, pore pressure and gene expression is calculated.

3.1.1. Vertical stress under a foundation

The vertical stress at any point in a soil, due to a point load at the surface can be calculated using Boussinesq's analytical solutions (Boussinesq 1885). These solutions assume a homogeneous, isotropic, weightless and elastic solid material, in an infinitely large half-space which is free of initial stress and deformation. Furthermore, the modulus of elasticity may be assumed to be constant and the principle of linear superposition is assumed valid. In the case of a rectangular raft foundation, integration of Boussinesq's solutions, as derived by Fadum (1948), gives the value of the vertical stress at the corner of the rectangle, assuming the pressure applied is uniform, normal and applied at the surface of the soil. Therefore, in order to calculate the vertical stress at any point beneath the raft foundation, it must be divided into four rectangles and the principle of superposition used to find the stress at the desired point (Fadum 1948). This is illustrated in figure 2, where P is the point of interest and the contribution of each rectangle is given by an *influence value* (I), calculated using equation (1) below (Fadum 1948, Poulos and Davis 1974).

$$I = \frac{1}{2\pi}\left[\arctan\left(\frac{m*n}{\sqrt{m^2+n^2+1}}\right) + \left(\frac{m*n}{\sqrt{m^2+n^2+1}}\right)\left(\frac{1}{1+m^2}+\frac{1}{1+n^2}\right)\right]. \quad (1)$$

In equation (1),

$$m = \frac{b}{z} \quad (2)$$

$$n = \frac{l}{z} \quad (3)$$

where b is the breath of the resulting rectangle once the foundation is split into four, l is the length of the resulting rectangle and z is the depth at the point of interest (see figure 2).

The vertical stress at point P due to the external loading applied is then:

$$\sigma_z = q * (I_1 + I_2 + I_3 + I_4) \quad (4)$$

where q is pressure over the foundation.

3.1.2. Excess pore pressure dissipation

The duration and spatial distribution of excess pore water pressure is important for this study as the proposed system depends on the soil maintaining elevated pore pressures long enough for the bacteria to detect and respond to high pressure levels. Values of excess pore pressure over time are calculated using Terzaghi's one-dimensional (1D) consolidation equation (Terzaghi 1943) (equation (5)).

$$\frac{\partial^2 u}{\partial z^2} c_v = \frac{\partial u}{\partial t} \quad (5)$$

where u refers to the excess pore pressure at the time t and depth z and c_v is the coefficient of consolidation, a measure of the rate at which the consolidation process proceeds, and is expressed as

$$c_v = \frac{k}{\rho_w \cdot g \cdot m_v} \quad (6)$$

where k is the permeability of the soil, ρ_w is the water density, g is gravitational acceleration and m_v is the coefficient of compressibility.

Terzaghi's 1D consolidation equation is solved using a mathematical solution in terms of Fourier series (Taylor 1948). A simplified version of this is used (equation (7)) in order to easily implement the differential equation in a coding environment (Abid and Pyrah 1988).

$$u_{z,\ t+\Delta t} = u_{z,t} + \beta * [u_{z-1,t} + u_{z+1,t} - 2 * u_{z,t}] \quad (7)$$

where u is the pore water pressure at depth z and time t, and β is expressed as follows:

$$\beta = \frac{c_v * \Delta t}{\Delta z^2} \quad (8)$$

where c_v is the coefficient of consolidation, as given by equation (6).

The consolidation equations described above rely on several assumptions:

- The voids of the soil must be completely filled with water.
- Both water and soil grains are perfectly incompressible.
- Darcy's law applies, i.e. the flow of water being squeezed out of the soil depends on the permeability of the soil and the hydraulic gradient in the direction of the flow (Darcy 1856).
- The coefficient of permeability k, is constant.
- The drainage of water is only vertical, i.e. 1D consolidation applies. Although this is not true in real situations, the results obtained with the computational simulations are accurate enough and give an insight into the magnitude and duration of excess pore pressures.
- The excess pore water pressure at the drainage surface(s) after the initial load is applied is equal to 0.

3.2. Integrated simulation

Using the models of soil stress and pore pressure described in the previous section, we can then map values of gene expression for individual bacteria within the soil, given expression values for a pressure sensitive gene. Enzyme activity in this instance is described in terms of fluorescence. This allows us to integrate actual data from our *in vivo* experiments (Guyet *et al* 2018) where we have used a gene which encodes for GFP to visualise the activity of our pressure sensing gene. In practice, we imagine that our genes of interest are either producing a product to bind soil grains (for example initiating biomineralisation or producing polymers) or a chemical which signals to another bacterium which, in turn is responsible for the synthesis of a material. To this end we have modelled variations of two scenarios:

1. In Scenario 1 we have a single bacteria type (Device 1) with a gene promoter which causes the production of a bio-cement in response to pressure.
2. In Scenario 2 the pressure sensing bacteria (Device 1) produces a signalling molecule which is received by another bacteria type which, in turn, is responsible for the material synthesis (Device 2). In this scenario, Device 2 is under the control of a promoter which is sensitive to signalling molecule produced by Device 1.

The model calculates values for pore water pressure throughout the soil volume at a given time, maps these values to predicted gene expression for the pressure sensing gene at that time and displays a matrix of boxes, the sizes of which are proportional to the gene expression we would expect to see at different points within the soil. Using this model, we can produce 2D sections through the soil volume, indicating zones of high gene expression, which correspond to cemented zones of soil, as shown in figure 3.

4. Results

A 10 m × 10 m × 10 m volume of soft clay was modelled with a constant uniform load of 1 MPa applied at the surface and a coefficient of consolidation of the soil, $c_v = 3\ m^2\ yr^{-1}$. This value was chosen to be representative of a typical clay soil (Terzaghi and Peck 1967). Two-way drainage was assumed, where water can drain from the top and bottom surfaces of the soil. All results shown are taken from the model soil 3 h after loading, to coincide with the biological test conditions in our connected study (described in section 2.1.2).

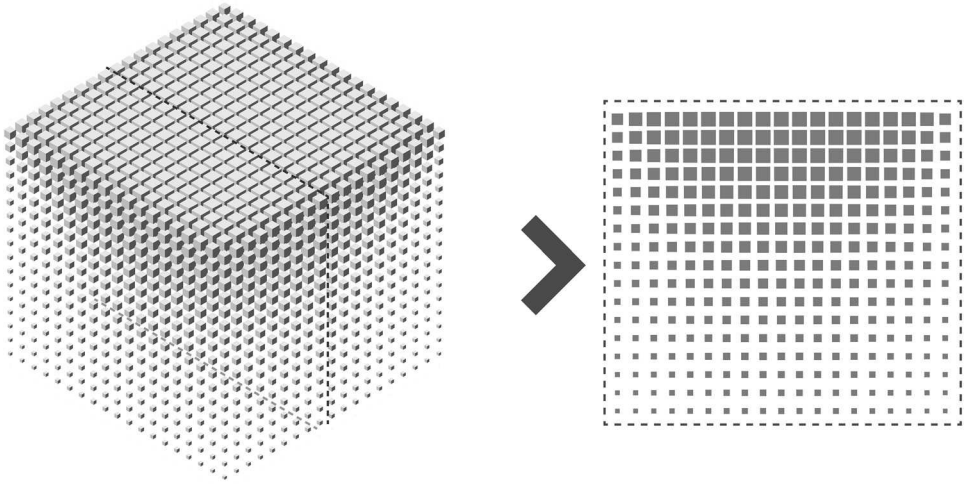

Figure 3. Output of our synthetic bio-cementing simulation with a 1000 m^3 volume of soil evenly loaded and divided into 6859 voxels. The voxel size is proportional to the gene expression in a population of engineered bacteria. The full volume is sectioned through the centre to produce a 2D sectional map.

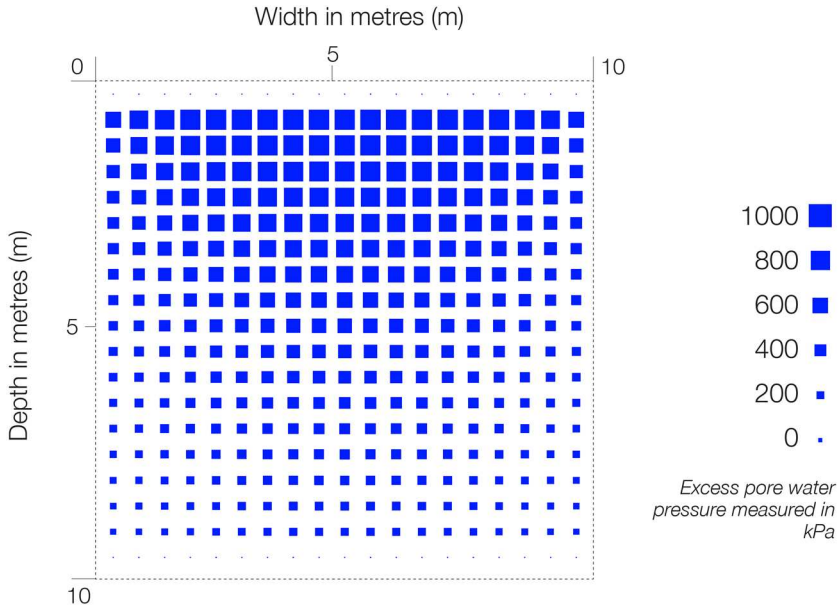

Figure 4. Visualisation to show excess pore pressure over a section of 1000 m^3 of soil loaded with 1 MPa. The section is taken midway through the soil volume and the grid of squares is drawn proportional to excess pore pressure 3 h after the load initially applied.

The pore pressures in the soil induced by the applied loading at this time point are shown in figure 4.

The following results are from modelling variations on two scenarios. In Scenario 1 we have single bacteria (Device 1) both sensing pressure and synthesising material in response. In this scenario we simulated a linear increase in the pressure response (figure 5(a)) and a specific sensitivity to a range of pressures (figure 5(b)).

We also applied our data from the *azuC-gfp* engineered strain (shown in table 1) both in terms of absolute expression (figure 6(a)) and as relative increase in expression to indicate the change more clearly (figure 6(b)).

Scenario 2 is based on a two-device system. In this scenario, the first bacteria (Device 1) has a pressure sensing promoter connected to a gene which codes for a signalling molecule and Device 2 constitutes a cell with a promoter that is sensitive to the signalling molecule expressed by Device 1. We describe Device 2 as the actuator device. Known signalling systems include for example subtilin which is used to signal between *B. subtilis* bacteria (Bongers *et al* 2005). We took the data obtained from the *in vivo* experiments on our pressure sensing gene (Guyet *et al* 2018) and input this profile for the signalling device. This signal is then picked up by a second actuator device. Figure 7 shows three different hypothetical actuator profiles and the potential patterns of gene expression in the soils.

Finally in a series of independent simulations, we applied loads of 0.1 MPa to 1 MPa to the volume of soil, in increments of 0.1 MPa and used the profile of the *azuC* gene to sense pressure changes in conjunction

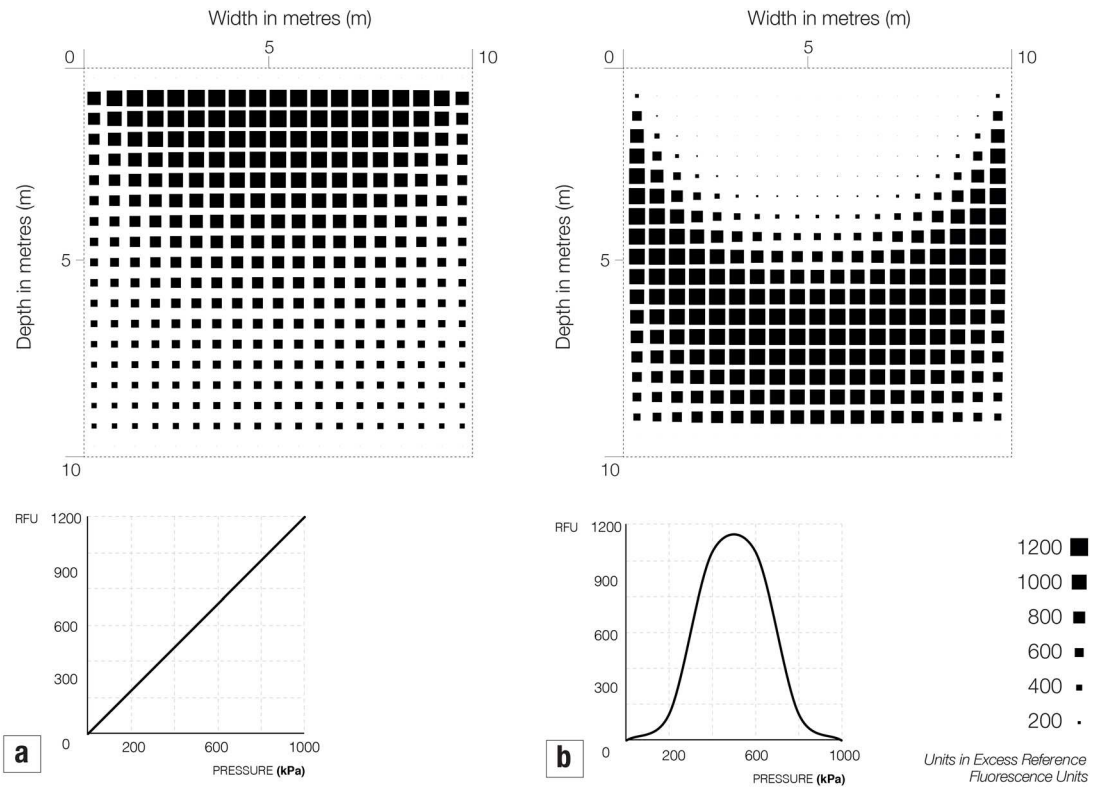

Figure 5. Visualisations to show gene expression values over sections of 1000 m^3 of soil loaded with 1 MPa. The sections are taken midway through each soil volume and the grid of squares is drawn proportional to the enzyme activity from two hypothetical pressure sensitive promoters where (a) has a linear increase in pressure response and (b) shows a sensitivity to a range around 500 kPa.

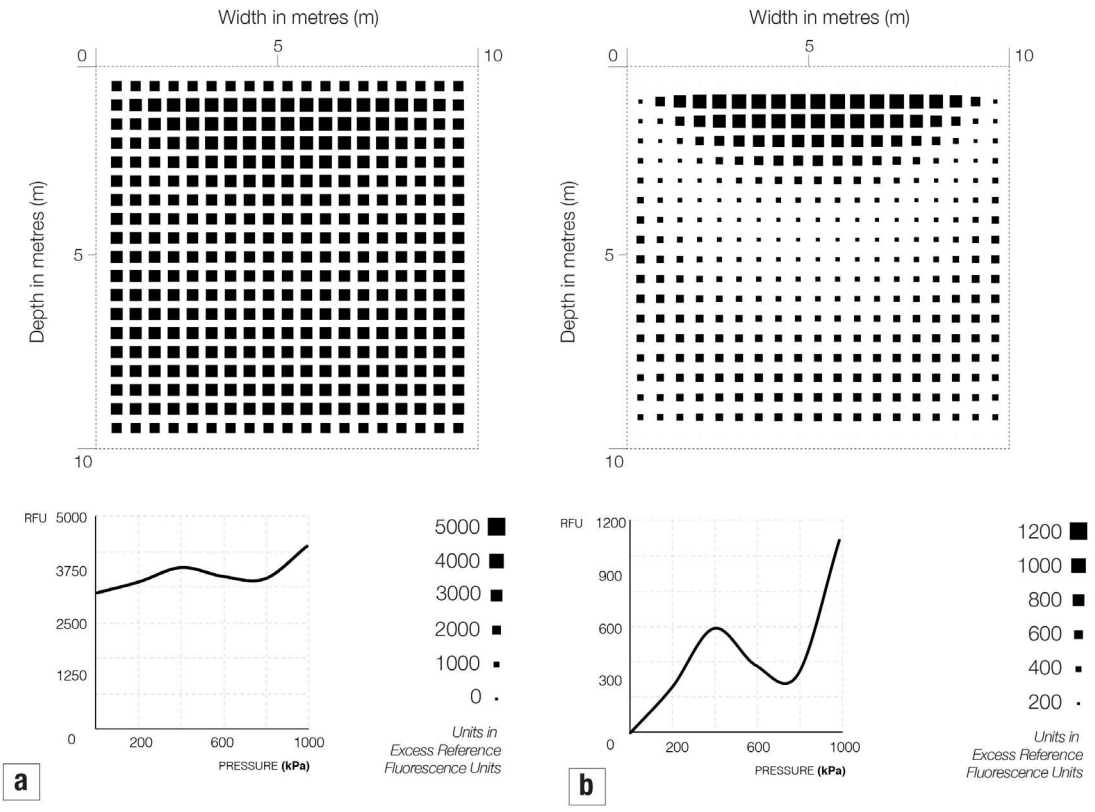

Figure 6. Visualisations to show gene expression values over sections of 1000 m^3 of soil loaded with 1 MPa. The sections are taken midway through each soil volume and the grid of squares is drawn proportional to the enzyme activity for the pressure sensitive gene *azuC* using (a) absolute values and (b) excess expression.

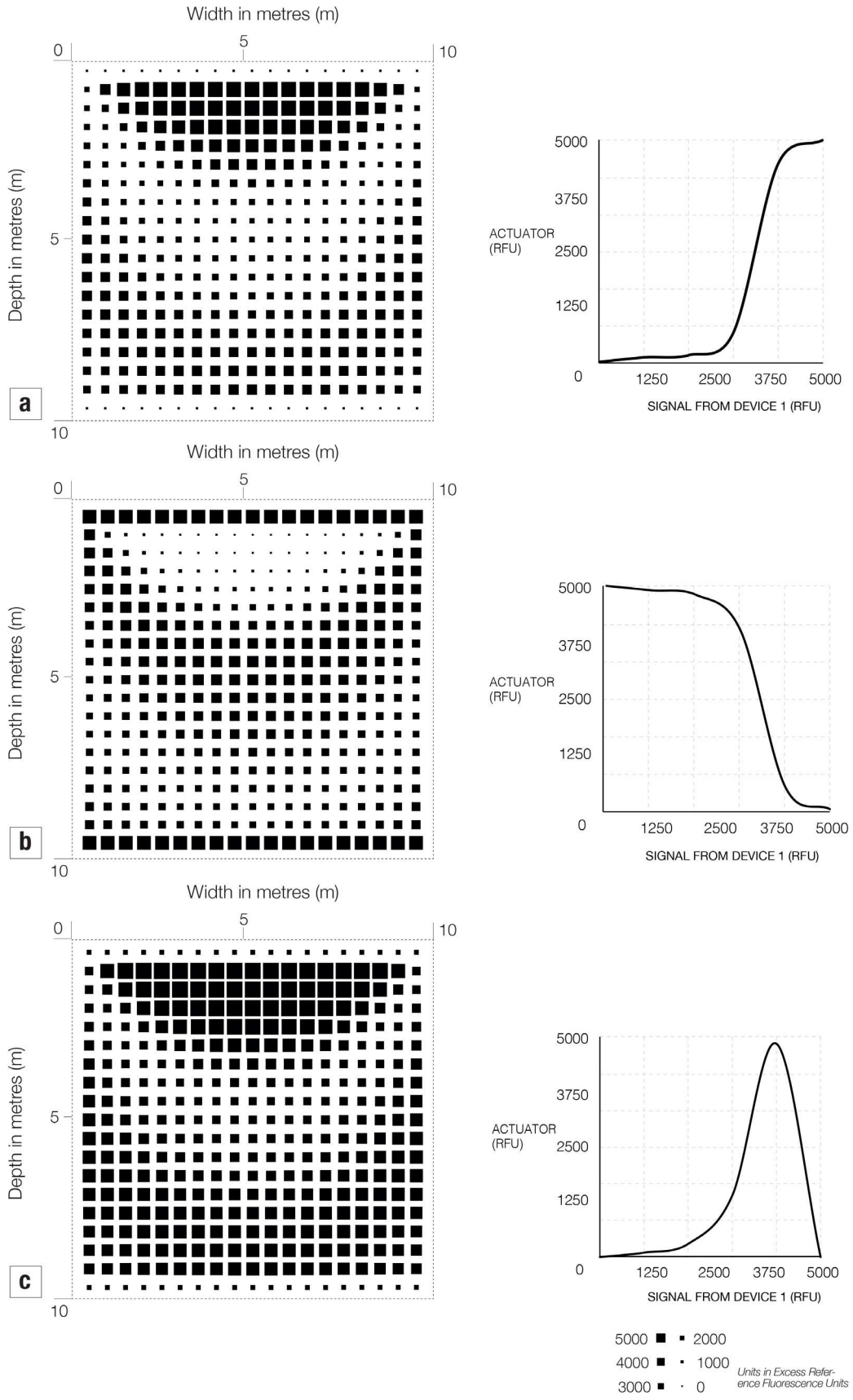

Figure 7. Visualisations to show gene expression values over sections of 1000 m^3 of soil loaded with 1 MPa. Sections are taken midway through each soil volume and the grid of squares is drawn proportional to the enzyme activity for three hypothetical promoters under the influence of a signalling molecule whose expression is mapped onto the profile for the pressure sensitive gene *azuC*. In (a) the actuator is sensitive to signals of high values, in (b) the actuator is sensitive to signals of low values and in (c) the actuator is sensitive to signals with a range of values around 3750 RFU.

with the signal sensing promoter profile (the actuator) from the example shown in figure 7(c). The results are shown in figure 8.

5. Discussion

The computational model described in this paper illustrates a new type of design process which integrates the nano behaviour of individual bacteria cells in terms of gene expression and the macro behaviour of soils under load. Figure 5(a) shows the expected pattern of expression for a hypothetical promoter where gene expression is linearly increased in response to elevated pressure. In this case, the highest expression occurs in the areas of highest pressures. We therefore might expect that, if this gene profile was connected to a process of material synthesis, most bio-cementation would occur where the stresses in the soil were greatest, which would be ideal for civil engineering applications. However, we are very unlikely to find this situation. In Kato *et al* (1994) for example, a pressure sensing promoter in *E. coli* was discovered which has a specific sensitivity range where increased expression was triggered at a relative narrow range of pressures (albeit at higher elevated pressure than we are interested in). In our own gene of interest *azuC* we also see a non-linear pressure responsiveness with a gene which is constitutively expressed irrespective of pressure and shows a sensitivity to specific ranges. The model allows us to visualise the implications of these complex relationships between elevated pressure and gene expression and, as we begin to develop a potential library of pressure sensitive genes, to select appropriate sensitivity profiles for a given context.

5.1. Integration of biological data into the computational model

We have also run the simulation with real biological data. In figure 6 we show two visualisations: one with absolute experimental data where the gene of interest is expressed even at 0 kPa of elevated pressure and a version with relative data which only shows elevated expression with the expression of the control set as zero. We see two bands of increased expression associated with the two peaks at approximately 400 kPa and continually rising to 1 MPa. If we associate these values with levels of cementing in the soil we would expect a zone of high cementation at the top of the soil volume with a crescent shaped void associated with low levels of expression below and then another region of increased cementation lower in the soil. Although this is not optimal from a civil engineering perspective, cementation is still achieved at the top of the soil volume where it is most required.

In our final visualisations we show the response of the *azuC-gfp* gene fusion as part of a two component system in which one cell signals under the control of the pressure sensor to another cell which, in response to the signalling molecule initiates material synthesis. There are two reasons why such a system may be implemented. First, engineering bacteria to make additional enzymes (such as signalling molecules, polymers or enzymes associated with biomineralisation such as Urease) places a metabolic load on the bacteria (Glick 1995) and may impede their function and survivability. By separating sensing and material synthesis into two separate cells, therefore, we spread the metabolic load between two separate systems. Secondly, separating the two functions creates two gene expression profiles. Bacteria containing Device 1 has a sensitivity to pressure and bacteria containing Device 2 has a sensitivity to the sensing product of Device 1. By combing different profiles, we can create different patterns of cementation in the soil. In our example in figure 7 we use the pressure sensing profile of the *azuC* gene fusion for the signalling bacteria and combine these with three different hypothetical profiles for our actuator bacteria. Combined with different profiles we see that different patterns of cementation may be generated in the soils. We can use this information to design an appropriate system by selecting an appropriate actuator for a given load condition. Figure 8 shows, for example, the pattern of gene expression under a number of different loading conditions. These visualisations show that the actuator amplifies the signal from the *azuC* gene and will perform optimally at between 100 kPa and 400 kPa before the effects of the decreased expression. In this case our simulation can be used to design an optimum actuator which effectively amplifies the signal for the pressure ranges we are interested in and mitigates the uneven nature of the pressure sensitivity of our *azuC* gene.

5.2. Limitations and future work

While the integrated computational model is novel and based on real (although preliminary) biological findings, the contexts we are using are abstract and still some distance from a real geotechnical scenario. The application of the biological data and integrated modelling should therefore be seen as illustrative of the potential of the system rather than properly predictive.

The model uses biological data from bacteria grown in a highly-controlled lab environment at optimum temperatures and with rich sources of nutrients which would not be found in soils. The genetic responses in a real soil environment would likely be significantly changed as the bacteria deal with less favourable growing conditions and competition from other organisms. In addition, within the *azuC* gene we are likely to be observing indirect effects of pressure which can be caused by other factors. Our aim now therefore, is to isolate a gene promoter which is specifically associated with pressure. We also see a high level of constitutively expressed GFP in our demonstrator system, i.e. relatively high levels of GFP are being detected when no pressure is applied. If we were to continue with this system, therefore, we would need

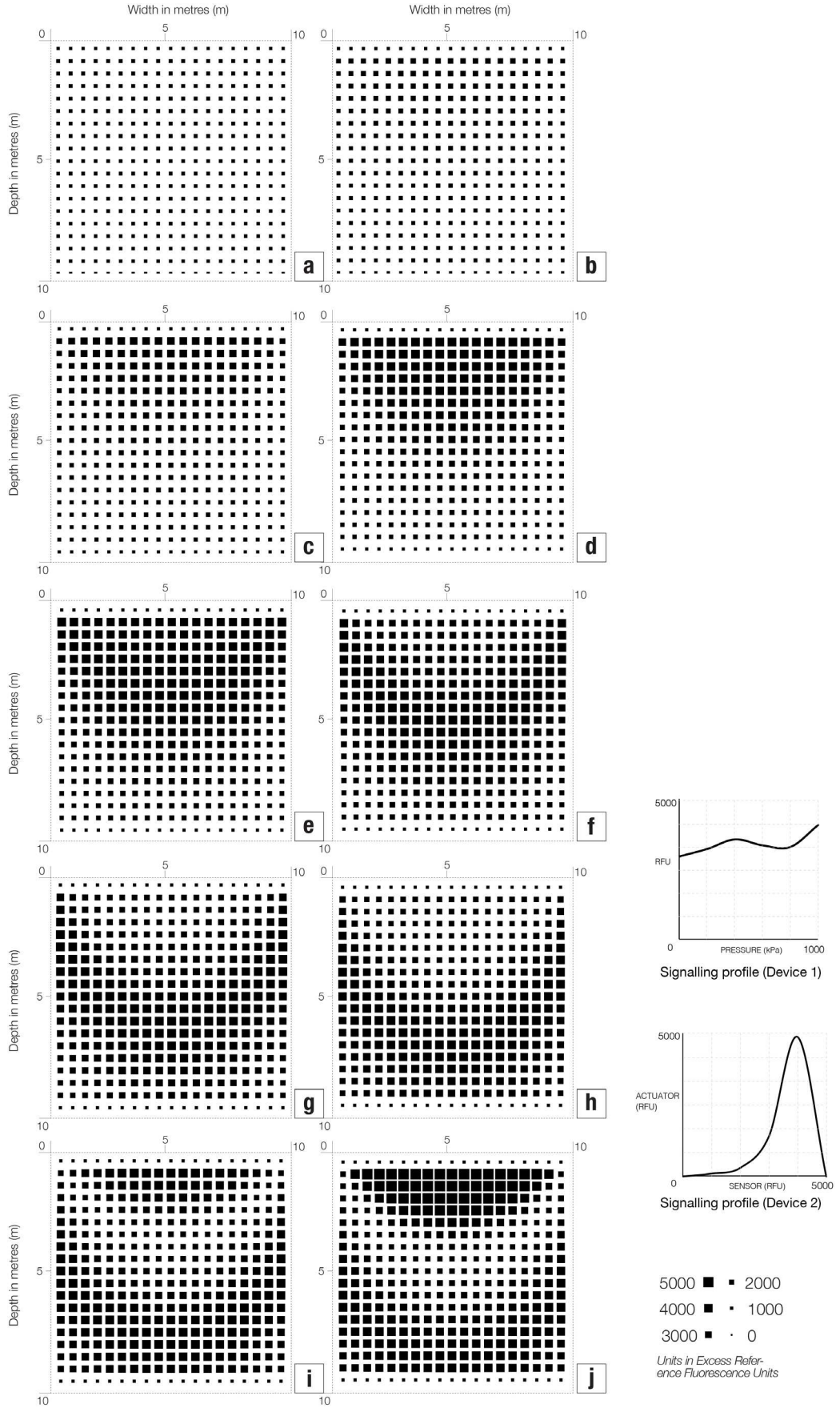

Figure 8. Visualisations to show gene expression values for over sections of 1000 m^3 of soil loaded with pressures from 0.1 MPa (A) to 1 MPa (J). Sections are taken midway through each soil volume and the grid of squares is drawn proportional to the enzyme activity for a number of hypothetical promoters under the influence of a signalling molecule whose expression is mapped onto the profile for the pressure sensitive gene *azuC*.

to consider whether the promoter could be refined to be more pressure specific. We also recognise that, while *E. coli* represents a useful model bacterium, it is not adapted to life in the soil. Developing our system will require the use of a bacteria species such as *B. subtilis* which is found living naturally in soils and also has the capacity for bio-cementing.

The computational model assumes a homogenous consistency of soil throughout the volume and an even distribution of bacteria. In a real geotechnical context we would expect highly heterogeneous soil properties as well as much greater bacterial cell activity at the surface soil layers where there is best access to nutrients. The model does also not account for the micro-scale physics of transporting molecules of the bacteria cells (signalling molecules of enzymes responsible for material synthesis) or the micro forces which would begin to affect the cells as materials are made. This biological behaviour is also time dependent and, at the moment, our model takes a snapshot at a point in time rather than considering time dependent biological processes including variability of gene expression, cell growth and cell death. In addition, the values for pore pressure used are obtained from standard formulas based on soils which are saturated with water. A bacteria mix is likely to be thicker than water and therefore the liquid flow through the soils will be slower—thus maintaining higher pressures in the soil for each condition longer. This also has an implication for potential applications. In this instance, the proposal is based on a notion that bacteria would be seeded into an existing volume of soil. This seeding process would likely involve the injection of a liquid culture of bacteria into the soil under high pressures through, for example, a deep irrigation system. Pressures associated with injecting the liquid through the soil must be taken into account because the pressure sensitive bacteria may become prematurely active when high pressures are being used. However, if fine-grained soils with a low coefficient of permeability are used it could take month or even years to properly irrigate the soil at low pressures.

These limitations aside we have succeeded in integrating biological data and geotechnical models—illustrating the relationships of multiple scales and beginning to implement a new type of design framework which accounts for these relationships.

6. Conclusions

The integration of *in silico* and *in vivo* work across multiple scales of system design is novel and integrates design processes for synthetic biology and geotechnical engineering, which have not been combined before.

The computational (*in silico*) work has approached the problem at the macro scale relevant to human construction. The key contribution here has been to integrate geotechnical behaviour with biological data in a general model which can be refined and adapted to explore the behaviour of a pressure-responsive bio-cementation system. In the computer model, changes in gene expression have the potential to lead to substantial changes in the pattern of bio-cementation in the soil. Even small changes in promoter sensitivity might fundamentally change the behaviour of the system at the macro scale. Overcoming this challenge but also exploring its potential could become an important part of the design process.

There is the potential for a great deal of further work in analysing the gene expression data and in developing the computer model. A key challenge is to develop an understanding of this system at intermediate scales, including factors such as the likely distribution of bacteria cells in the soil. As the work moves towards an implemented physical demonstrator, a better understanding of the behaviour of bacteria cells as colloids within different types of soil is necessary, as well as the development of models which include data on the chemical composition of soils which are likely to have a radical effect on the outcome of such a system.

While there are limitations in the work described here, it illustrates a new design process in which the analysis of soil conditions leads to the design of micro-organisms to improve soil stability and strength and even cause specified patterns of cementing. As our *in vivo* work develops we should be able to plot promoter sensitivity for a selection of our known pressure sensitive genes and, in the future, we may be able to edit promoters to give us the desired sensitivity profile—sculpting material responses to pressure by altering sequences of DNA and through the interaction of many different genetic devices and engineered organisms.

Acknowledgments

This work was supported by the UK Engineering and Physical Sciences Research Council, grant number EP/N005791/1.

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