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Agarose gel as a soil analogue for the development of advanced bio-mediated soil improvement methods

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Abstract

Bio-mediated soil improvement methods (those that use biological processes) have potentially low cost and environmental impact but can be difficult to control to ensure effective results, especially if engineered bacteria are used. A novel application of using agarose gel as a soil analogue is proposed, which can enable development of advanced bio-mediated soil improvement methods by reproducing relevant mechanical properties while allowing complex biological processes to be studied in detail, before testing in soils. It is envisaged that agarose gel will be used instead of soil when developing early-stage prototype methods, as it provides an ideal environment to facilitate growth and monitoring of bacteria. A programme of geotechnical tests and Scanning Electron Microscopy on Agarose Low Melt (LM) gel is presented. The results demonstrate comparable pore size, undrained strength and permeability to soft clays and peats but more linear stress-strain behaviour and higher compressibility. This paper offers proof of this novel concept but further investigation is required as only a single type of agarose, at a single concentration is tested. By varying these factors, along with use of different solvents, there is significant potential to tune the behaviour of the analogue to particular soils or construction scenarios.

Key words

Hydrogel, Agarose, Soil Analogue, Soil Improvement, Bio-mediated, Biological

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Introduction

Bio-mediated soil improvement techniques use biological processes to improve soil properties. *Biocementation* can strengthen soils (Whiffen et al. 2007, Ivanov and Chu 2008, Martinez et al. 2014), *biodesaturation* reduces soil saturation through the production of biogas (Chu et al. 2009, DeJong et al. 2013) and *bioremediation* can remove contaminants (White et al. 1998, Stabnikov et al. 2015). These techniques have a wide range of applications and potentially low cost (White et al. 1998, Horemans et al. 2017) and environmental impact (DeJong et al. 2009, DeJong et al. 2010).

However, despite the potential of bio-mediated methods, there is still significant multidisciplinary research required to optimise and develop these complex processes, to provide more control and certainty of outcome. It is also possible to advance bio-mediated methods further by using Synthetic Biology approaches to engineer biological systems so that their properties and response to external stimulus can be controlled (Endy 2005). Synthetic Biology allows design of living organisms by altering their genetic circuits, enabling them to sense their environment and respond in ways that they would not do naturally. For example, in a previous study the authors have shown how engineered bacteria can be developed to respond to elevated pressure (Dade-Robertson et al. 2018) with the objective of then engineering this bacteria so that it is able to respond to elevated pressure by synthesising material. An application of this would be a responsive bio-mediated ground improvement method which would enable a soil to increase its strength when loaded (Dade-Robertson et al. 2018).

Biological systems are complex and sensitive, so a high degree of control over testing conditions is required for the molecular level manipulation of living cells in the early development of a prototype bio-mediated soil improvement method. It is therefore helpful to have a soil analogue which has similar relevant mechanical properties to soils but which allows easier monitoring, greater control of the chemical environment and also minimises risk of contamination from, for example, other microorganisms. Although these will be present in real soils, at the early development stage it is advantageous to minimise complexity. It is proposed that agarose gels can provide such a soil analogue for the early development of advanced bio-mediated soil improvement techniques – a novel

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concept which has not been previously proposed. This type of gel has a porous structure comparable to cohesive or organic soil, although it is fibrous rather than granular. The gel also allows controlled simulation of a variety of chemical, physical and mechanical properties. These gels are already used routinely in microbiology for culturing and monitoring bacteria so provide ideal conditions for this.

Agarose gels have been extensively studied at small scale for a wide range of applications including biomedical applications, tissue engineering, drug delivery, soft electronics and actuators (Zhang and Khademhosseini 2017, Varaprasad et al. 2017, Ionov 2014) but little information exists about their behaviour and properties at a macro-scale relevant to civil engineering. This paper describes a program of geotechnical experimental testing to determine the strength, stress-strain behaviour, permeability, consolidation behaviour and details of the microstructure of Agarose LM gel. It should be noted that Agarose LM gel is not added to soil under any circumstances and the properties reported are for the pure gel only. These properties are then compared to those of cohesive and organic soils, to assess the suitability of agarose for the novel application of a soil analogue for the early development of advanced bio-mediated soil improvement methods.

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Materials and methods

Materials

Agarose gel

Agarose is extracted from one of several types of marine red algae and is one of the main components of agar. Agarose gels consist of a network of fibres held together by non-covalent hydrogen bonds and microvoids holding water (Stellwagen and Stellwagen 1995). The mechanical properties of the gels are mainly dictated by the fibre-pore structure which depends on several factors including agarose type and concentration (Narayanan et al. 2006). 2-Hydroxyethyl Agarose, or Agarose Low Melt (LM) supplied by Melford Laboratories was used in all experiments.

Solvent

A solution of LB Broth (Miller), provided by Sigma Aldrich (components: 10g/L Tryptone, 10g/L NaCl and 5g/L Yeast Extract), dissolved in distilled water was used where possible to prepare the samples. If the agarose gel is used to develop bio-mediated soil improvement methods as intended, a nutrient broth such as this will be necessary to enable bacteria growth. Therefore, LB broth was chosen as the solvent to give mechanical properties which are as representative as possible for this application. However, for the consolidation tests which were of much longer duration (several weeks), only distilled water was used to avoid the growth of unwanted bacteria in these cases. It is possible that the change in solvent and even growth in bacteria may affect the mechanical properties of the agarose gel however this is outside the scope of the current investigation. Distilled water was also used in the preparation of the samples for Scanning Electron Microscopy, to avoid interference when imaging the structure of the gel.

Kaolinite

The kaolinite used to prepare the clay samples was Kaolin provided by IMERYS Ceramics.

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Experimental methods

In order to assess Agarose LM gel as an analogue for soil, a range of geotechnical experiments were performed. The experiments were chosen to determine the physical and mechanical properties of the gel of relevance to the behaviour of fine-grained soils. It was anticipated that the behaviour of the agarose gel would be most similar to that of clays due to the pore sizes of these types of soil and the electrostatic forces between clay particles which allow high water adsorption (Knappett and Craig 2012).

Visualisations of the structure of the gel made with different mass concentrations of agarose and also under load were obtained using Scanning Electron Microscopy (SEM). Analysis of these SEM images allowed estimates of pore size and porosity. The undrained shear strength (c_u) and stress-strain relationship were determined using Unconsolidated Undrained triaxial tests. In triaxial tests, the samples are subjected to a confining pressure or radial stress, σ_3 and an axial stress, σ_1 applied vertically. The permeability of the gels was also investigated using Isotropic Consolidation triaxial tests, where drainage of the pore water at different consolidation pressures is allowed. Permeability is particularly important as it affects the transport and distribution of nutrients and microbes through the porous structure. Finally one-dimensional consolidation of the gels was investigated using an oedometer.

Agarose gel sample preparation

The agarose gel was formed by dissolving Agarose LM powder in distilled water or LB media. This powder has low solubility in these solvents at room temperature, therefore the heterogeneous mixture was heated to 121°C for at least 30 minutes, stirring continuously in order to achieve a homogeneous solution. The solution was then immediately poured into aluminium moulds for triaxial or oedometer testing or 25mm Petri dishes for SEM.

For the triaxial tests, 38mm diameter cylindrical moulds were filled with gel to approximately a height-to-diameter ratio of 2. The cylinders were immediately covered with tape in order to avoid evaporation and then stored in a fridge at 4°C for approximately 15 minutes until gelation, with no

further curing required. For the oedometer tests, samples were prepared in the same way, although the moulds used in this case were 50mm in diameter and 20mm in height. Samples with mass concentrations of 2%, 4% and 6% m/v (mass/volume) were imaged using SEM and for all geotechnical tests, a concentration of 6% m/v was used. This was chosen after initial investigation determined that this was the highest concentration possible that allowed homogeneous growth of bacteria, which was required for associated studies on developing bacteria-based soil improvement methods.

Kaolinite sample preparation

Kaolin powder was thoroughly mixed with water until a homogeneous mixture was achieved. The mixture was then consolidated at 100kPa for a week. Cylindrical samples were cut from the consolidated clay using 38mm diameter moulds. The top and bottom of the cylinders were then covered with wax in order to avoid changes in the water content and stored in a cool environment until testing.

Saturation of samples

The method of making the agarose gel produces saturated samples. When the powder is mixed with the solvent and heated a dense liquid is formed and some bubbles are present. However during the heating process the solution is mixed continuously and these bubbles can be observed to migrate to the surface and disappear. Once a homogenous solution is achieved, no remaining bubbles are visible and the gels are assumed to be fully saturated. To confirm this is the case, the pore pressure coefficient, *B*, or Skempton's B-value (Skempton 1954) was obtained before starting all triaxial tests. To determine the B-value, increments of confining stress were applied and the increment in pore pressure measured. It was ensured that the B value was above 0.95, as recommended by British Standard testing methods (British Standards Institution 1990c) and also that the change in pore pressure was instantaneous with the change in confining pressure. B values for the kaolinite samples were also obtained in the same way.

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Scanning Electron Microscope imaging

The scanning electron microscope used was a field emission TESCAN MIRA 3. Agarose gel was prepared as described in 'Agarose gel sample preparation'. Upon gelation, 5mm^3 cubes of gel were cut and placed inside 10mL beakers. Liquid nitrogen was then poured into the beakers to guarantee rapid freezing of the samples and avoid structural deformations during the freeze-drying process. The use of ultrafast freezing techniques avoids distortions and deformations of the specimens' structure to as little as the nanometre scale (Robards 1991). The beakers were then placed into a vacuum cell and were freeze-dried under vacuum at -80°C for 24h. Finally, before SEM inspection, the samples were sputter-coated with a layer of platinum between 3nm and 4nm thick using a High Resolution Sputter Coater. The samples were visualised at a very low voltage (1.5kV to 2kV) in order to avoid any damage to the structure.

Triaxial tests

Two different series of triaxial tests were performed using a GDS 50kN digital load frame: Unconsolidated Undrained (UU) tests and Isotropic Consolidation (IC) tests.

For the UU triaxial tests, Agarose LM cylinders were produced as described in 'Agarose gel sample preparation' and then demoulded, prepared and tested according to British Standard testing methods (British Standards Institution 1990d). The samples were wrapped in an impervious membrane and confined between impervious end caps before being introduced into the triaxial cell. This allowed maintenance of a constant moisture content. The kaolinite clay samples were also demoulded and tested to failure following the same procedure.

For the IC triaxial tests, pressure-volume controllers were attached to the triaxial cell and connected to the top and bottom of the sample, in order to measure and control pore pressures and drainage. Special caps including porous stones were also placed top and bottom of the sample which allowed drainage. The tests were performed according to British Standard testing methods (British Standards Institution 1990c) at different effective stresses (25kPa, 50kPa, 100kPa and 150kPa).

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Oedometer tests

50mm diameter disks were prepared as described in 'Agarose gel sample preparation' and tested according to British Standard testing methods (British Standards Institution 1990b). Drainage was allowed from both top and bottom of the sample and filter paper was added between the sample and the porous stone to ensure that no gel entered the pores of the stone during the consolidation stage.

Agarose gel samples were consolidated anisotropically at different consolidation pressures (3kPa, 6kPa, 12kPa, 25kPa and 50kPa) by applying increments of axial stress. At the end of the consolidation test, the samples were unloaded in the same increments.

Results

Effect of concentration on microstructure

Fig. 1 shows SEM images of Agarose LM gel samples at different mass concentrations (2%, 4% and 6% m/v), at the same scale, at two different levels of magnification. It can be seen qualitatively that increasing gel concentration results in more densely packed fibres and reduced pore size. The pore diameters shown in each image in the top row of Fig. 1 were measured using ImageJ (an open-source image processing software) and the results are represented in Fig. 2. The reduced pore size at higher concentrations means that it is harder for bacteria to grow homogenously, as they are more constrained. This consideration governed the choice of concentration of hydrogel used for all mechanical tests (see section 'Agarose gel sample preparation').

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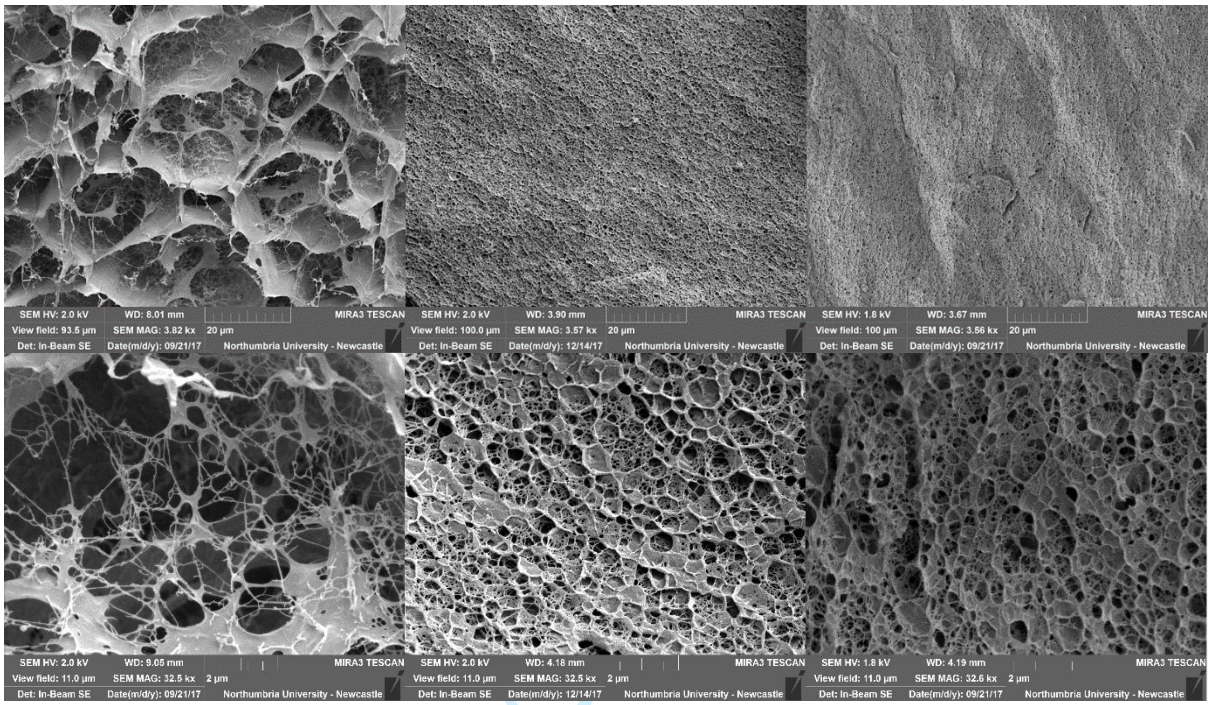
200 Fig. 1. SEM images of microstructure at mass concentrations of 2% (left), 4% (middle) and 6% (right).

201 The bottom row is at 10 times the magnification of the top row.

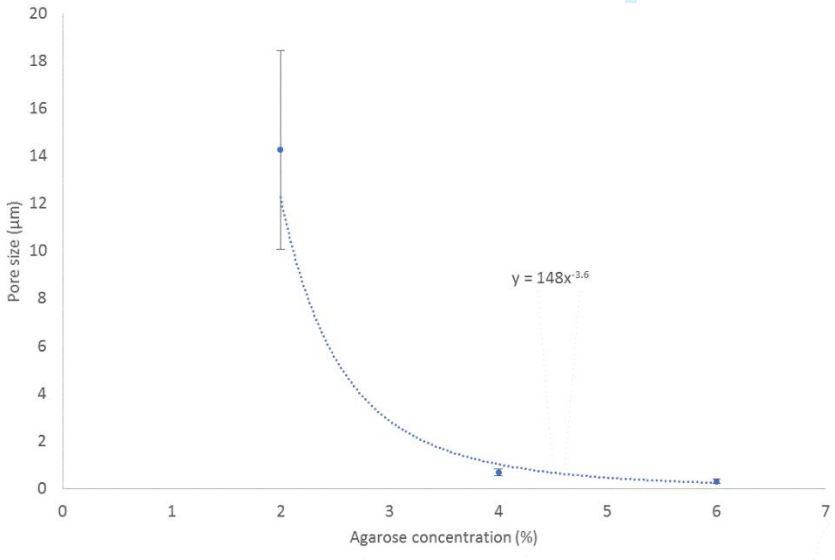
202



203

204

205 Fig. 2. Pore size as a function of agarose concentration.



206

207 The error bars in Fig. 2 show the variation of pore size at each concentration. Due to the

208 reduced size of the pores, this variation is not so clear for the higher concentrations in Fig. 2 but size

209 variation is still present, as can be seen qualitatively in the higher magnification images in Fig. 1. The

results indicate a relationship between the concentration of agarose *C* and the pore size, *a*, of the form:

(1) $a \sim C^{-\gamma}$

where *γ* is a constant that depends on the agarose type and the setting temperature (Narayanan et al. 2006) and is found to be 3.6 in this case. This result differs from those found by previous researchers, for example Ogston (1958) and de Gennes (1979) who give values of *γ* between 0.5 and 0.75. This difference may be related to the use of Agarose LM over standard agarose.

Porosity, void ratio and moisture content

Ogston et al. (1973) developed a method to determine the volume fraction of fibres ϕ, which can be calculated as:

(2) $\Phi = \frac{C}{\rho\omega}$

Where *C*, ρ and ω are concentration of agarose in the gel (m/v), dry agarose density and mass fraction of agarose in a fibre, respectively. The last two values can be estimated as 1.64 g/mL (Laurent 1967) and 0.625 (Johnson et al. 1995). From the volume fraction of fibres, ϕ , the porosity n and the void ratio *e* can be obtained with the following expressions (Pluen et al. 1999):

(3) $n = 1 - \phi$

(4) $e = \frac{n}{1-n}$

The moisture content can be also be calculated according to the following expression:

(5) $w = \frac{m_w}{m_{dry}}$

where m_w and m_{dry} are the mass of water and mass of dry solids, respectively.

The moisture content was also obtained experimentally for cylinders (50mm in diameter and 20mm in height) of agarose gel, according to British Standard testing methods (British Standards Institution 1990a). From this, an experimental void ratio can also be obtained with the relationship between void ratio, moisture content and specific gravity (G_s) for a saturated soil (Smith 2014):

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(6) $e = wG_s$

where w is the water content and G_s is the specific gravity of dry agarose (1.64g/mL as previously). Table 1 represents the theoretical and the experimental values obtained for the porosity, void ratio and moisture content of 6% m/v Agarose LM gel. The theoretical and experimental values of water content agree reasonably well and the difference between the values is likely to be due to the assumptions of dry agarose density and mass fraction of agarose in a fibre, which have not been measured for this particular Agarose LM gel.

Stress-strain relationship

The stress-strain relationship for samples at different confining pressures is presented in Fig. 3, along with an inset photo showing the failure plane of two Agarose LM samples. From this data the undrained shear strength, c_u, can be also derived according to British Standard methods (British Standards Institution 1990d).

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Fig. 3. Stress-strain relationship at different confining pressures, with inset of samples showing failure surface.

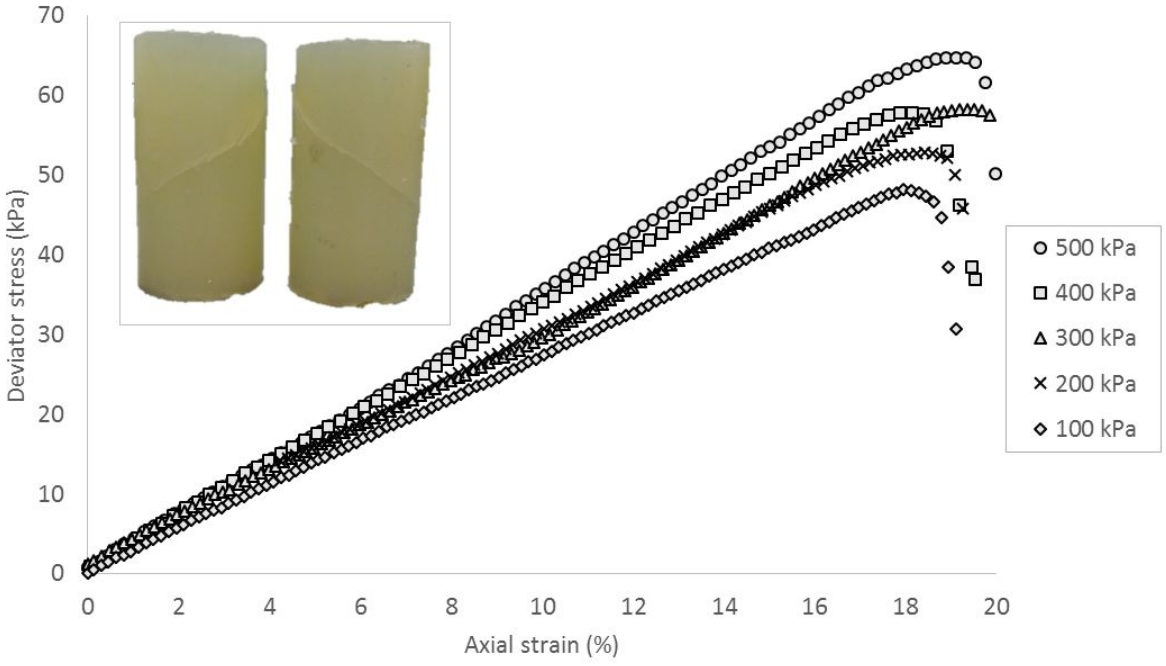
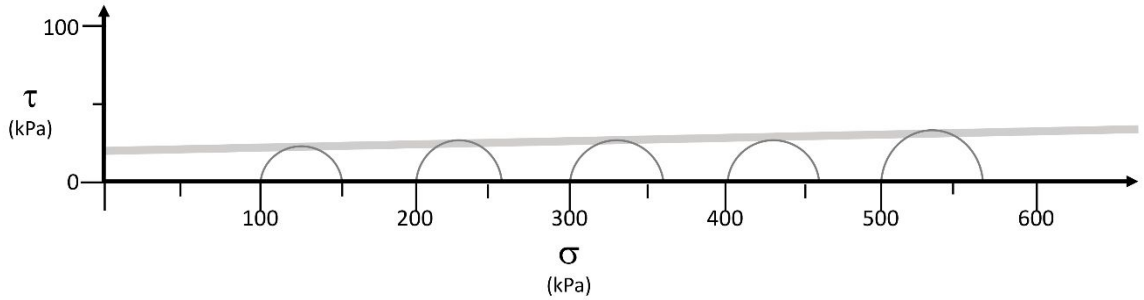


Fig. 3 shows that the maximum deviator stress increases with confining pressure (35.5% between the samples tested at 100kPa and 500kPa). The elastic modulus also increases with confining pressure (31.7% higher for the sample tested at 500kPa in comparison to the sample tested at 100kPa). The results for undrained shear strength, maximum strain and elastic modulus for each of the specimens are summarised in Table 2. An average of these values gives a shear strength of 27 kPa, a maximum strain of 18% and a modulus of elasticity of 309 kPa for Agarose LM gel. A plot of the data in the form of Mohr's circles is also shown in Fig. 4.

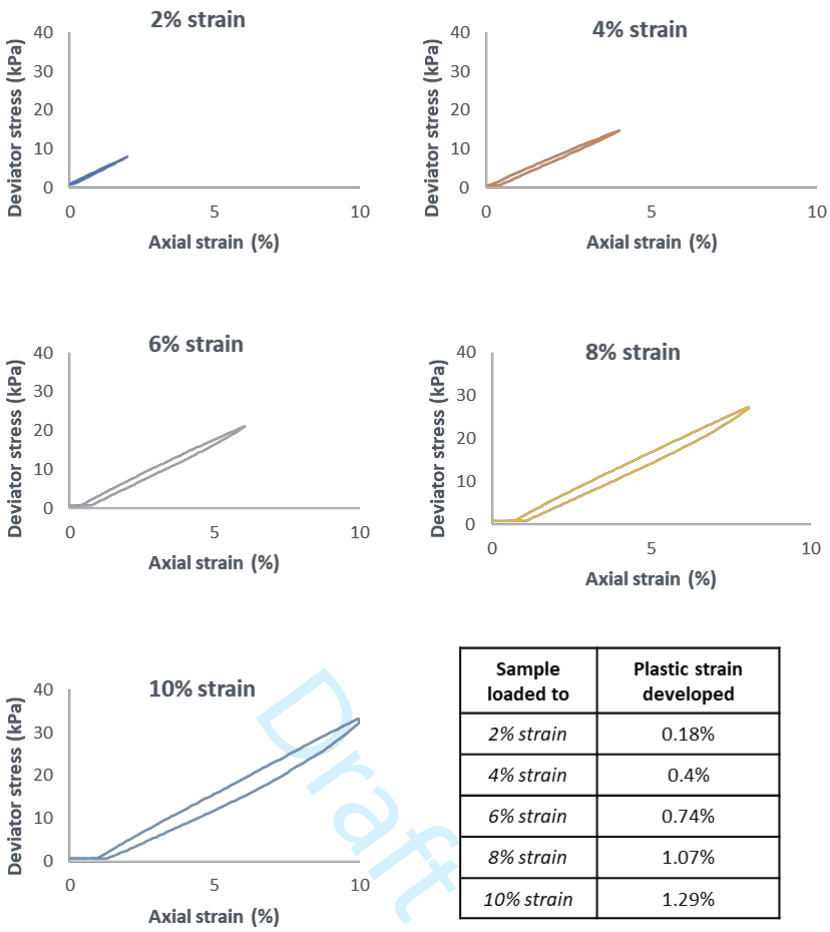
Fig. 4. Unconsolidated-undrained triaxial test results in the form of Mohr's circles.



From Fig. 4 it can be seen that a tangent to the Mohr's circles is not perfectly horizontal, also demonstrating the increase in undrained shear strength shown in Table 2. As the samples are fully saturated (see 'Saturation of samples'), a possible explanation of the increase in undrained shear strength with confining pressure is a small leak in the triaxial cell causing minor consolidation. If this is the case, without the leak it would be expected that the shear strength would not increase and would be of a comparable magnitude to the lower values obtained.

Agarose LM gel samples were also loaded to different axial strains (2%, 4%, 6%, 8% and 10%) under a confining pressure of 500kPa and immediately unloaded, as can be seen from Fig. 5.

Fig. 5. Loading and unloading behaviour at different strain levels.



Sample loaded to	Plastic strain developed
2% strain	0.18%
4% strain	0.4%
6% strain	0.74%
8% strain	1.07%
10% strain	1.29%

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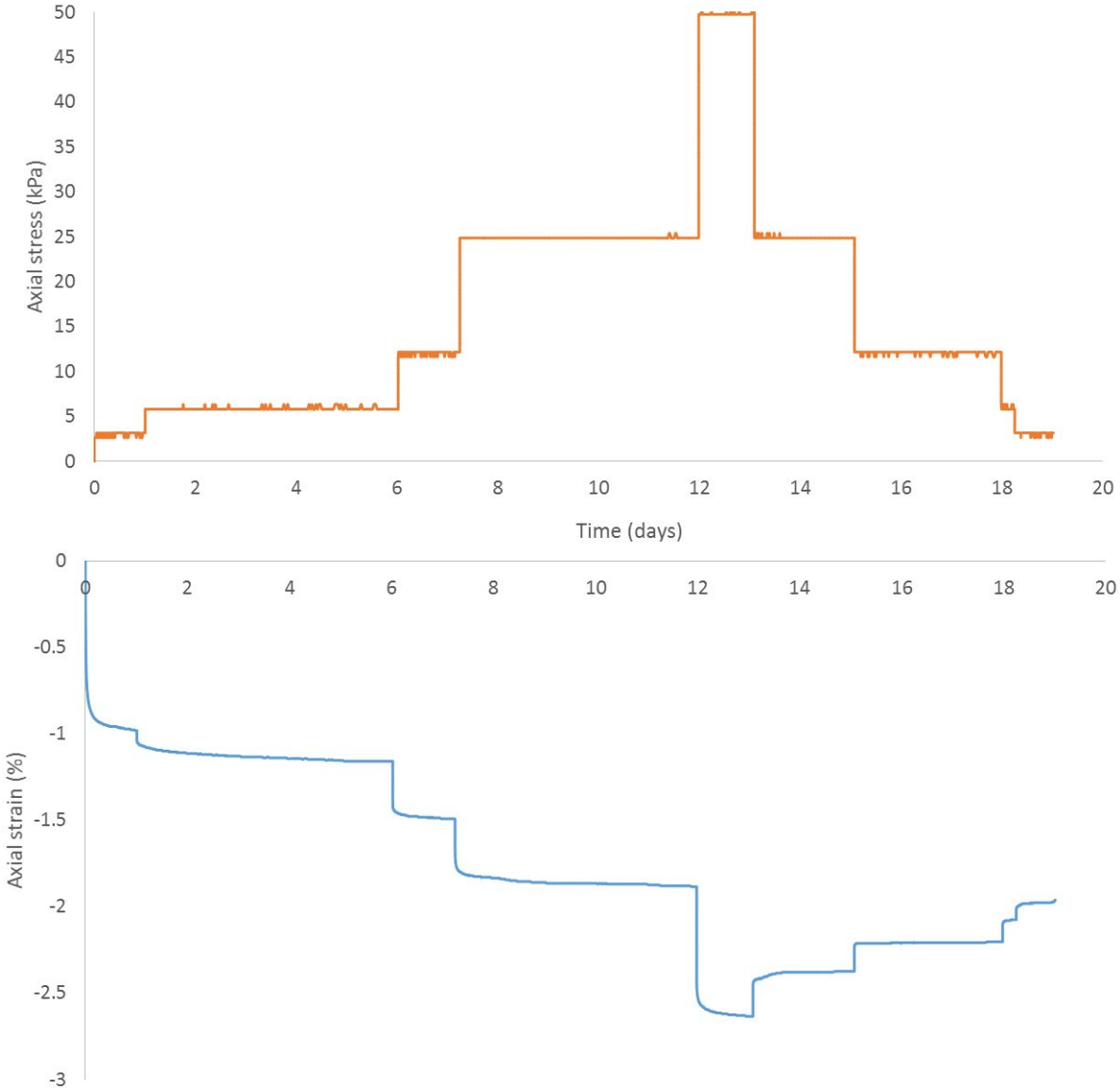
The residual/plastic axial strains developed after unloading are also summarised in Fig. 5. These results show that Agarose LM gel presents short term mainly elastic behaviour although some plastic strains do develop. Greater residual strains also develop at higher strain levels.

Anisotropic consolidation behaviour

For the samples tested in the oedometer cell (one-dimensional consolidation), the rate of change of axial strain in the samples decreased for every stage as consolidation occurred, allowing calculation of the coefficient of consolidation. Fig. 6 shows all the stages of anisotropic consolidation at 3 kPa, 6 kPa, 12 kPa, 25 kPa and 50 kPa, including both the loading and unloading stages.

287 Fig. 6. Anisotropic consolidation behaviour at different stress levels.



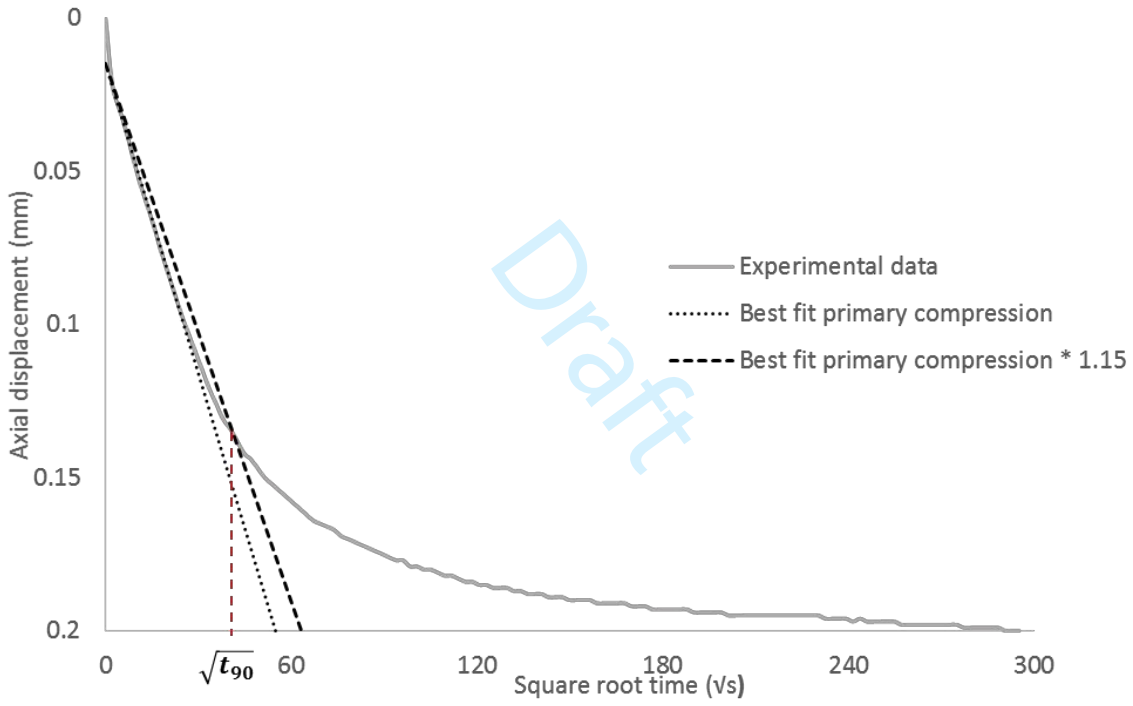
288

289 Taylor's method (British Standards Institution 1990c) for one-dimensional consolidation was
290 used to calculate the coefficient of consolidation, c_v for each loading stage. This method is based on
291 the approximation that the relationship between axial displacement and time is parabolic for degree
292 of consolidation < 60%, therefore the relationship between the axial displacement and the square root
293 of time is linear. Secondary consolidation is also assumed to be negligible for degree of consolidation
294 < 90%. Thus, c_v was calculated using t_{90}, according to the following expression (British Standards
295 Institution 1990c):

(7) $$c_v = \frac{0.446 * \overline{H}^2}{t_{90}}$$

where $\overline{H}$ is the average height of the specimen between the start and the end of the consolidation stage and t_{90} is the time for 90% of consolidation. Fig. 7 shows the steps used to derive the value of t_{90} for the first stage at 3kPa. The values of c_v for every stage are given in Table 3 and range between 0.89-3.87 m^2/year.

Fig. 7. Derivation of t_{90}.



Additionally, the coefficient of volume compressibility, m_v was calculated according to the following expression (British Standards Institution 1990c):

(8) $$m_v = \left(\frac{H_2 - H_1}{H_1}\right)\left(\frac{1000}{p_2 - p_1}\right)$$

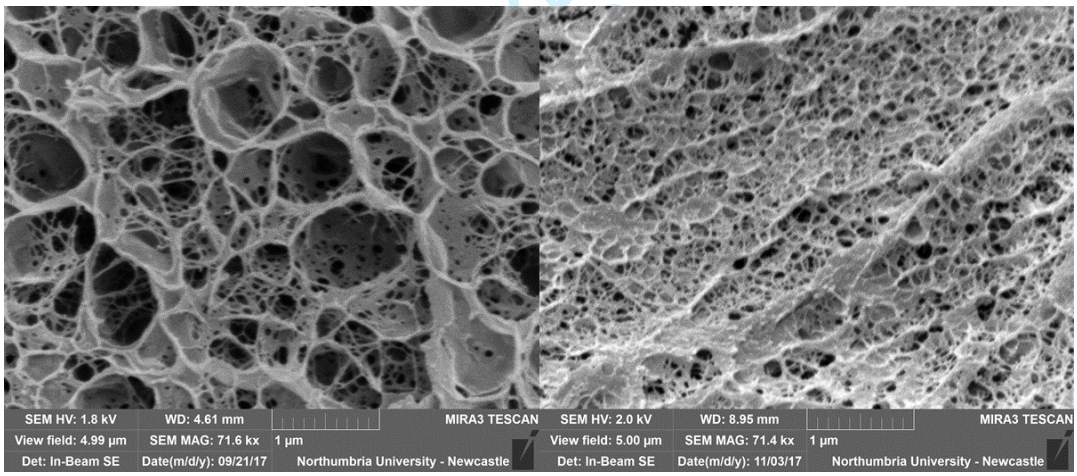
where H_2 and H_1 are the height of the specimen at the end and start of the load increment, and p_2 and p_1 are the pressures applied to the specimen for the previous and the considered loading stage,

respectively. The results for each consolidation stage are show in Table 4 and range between 0.3 and 3.26 m^2/MN.

Effect of consolidation on microstructure

$5\ \mathrm{mm}^3$ cubes were extracted from one of the samples isotropically consolidated for 21 days at 100kPa and were prepared for SEM inspection as described in 'Scanning Electron Microscope imaging'. Fig. 8 shows the microstructure of an unconsolidated sample and a sample consolidated for 21 days. The structure is denser and the sizes of the pores are smaller for the consolidated sample, due to the drainage of water during consolidation. The pore size was measured as described in 'Effect of concentration on microstructure' and decreases from 0.3 µm $\pm$ 0.09 µm when unconsolidated to 0.07µm $\pm$ 0.016 µm after 21 days consolidation. This reduction in pore size is expected to result in a consequent decrease in permeability.

Fig. 8. Microstructure of consolidated (right) and unconsolidated (left) Agarose LM gel.



Permeability

To estimate the vertical permeability, k_v the following expression derived from Darcy's law was used:

(9) $$k_v = \frac{1.63 * q * L * 10^{-4}}{A * (p_1 - p_2)}$$

where q is the mean rate of flow through the bottom of the specimen, L is the length of the specimen prior to testing, A is the area of the specimen prior to testing and $p_1 - p_2$ is the pressure difference, or consolidation pressure in this case. Thus, the vertical permeability k_v for 6% m/v Agarose LM gel ranges between $3.4\ x\ 10^{-11}$ and $8.8\ x\ 10^{-11}$ m/s depending on the effective stress applied, as can be seen from Table 5.

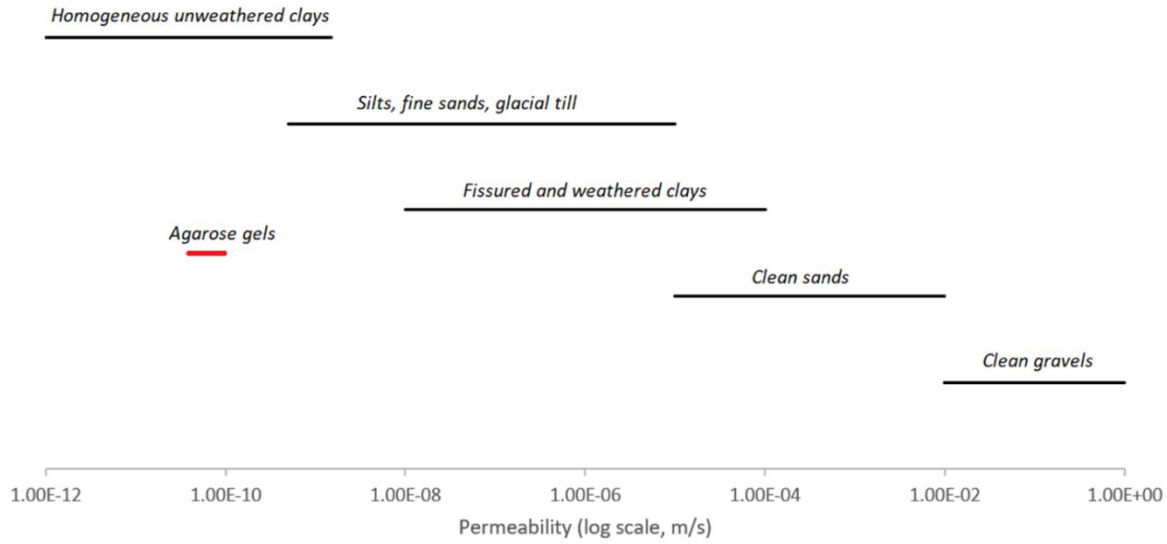
It should be noted that these results do not express directly measured values of the vertical permeability, but an estimation made from the amount of fluid drained from the samples. However, they provide a good indication of the permeability and allow a comparison with soils.

Discussion: comparison to saturated cohesive soils

Agarose LM gel has a fibrous microstructure that, although it is not granular, has a porous structure similar to fine-grained soils. An experimental investigation was performed in order to analyse the mechanical and physical properties of 6% m/v Agarose LM gel and Table 6 summarises the results and provides a comparison to saturated cohesive soils for each of the properties. It should be noted that while the type of behaviour is expected to be similar for other concentrations of agarose, the magnitude of properties may be different.

It can be seen from Table 6 that there are a number of similarities between Agarose LM gel and clays and peats, particularly the pore size, coefficient of consolidation and permeability values (as shown more clearly in Fig. 9). The permeability of Agarose LM is expected to be higher in specimens produced with lower concentrations of agarose (Narayanan et al. 2006, Pernodet et al. 1997), placing it towards the higher end of the range of clay permeability (Fig. 9). The undrained shear strength and undrained behaviour is also comparable to clays and can be analysed in similar way, as shown in Fig. 4. Additionally, SEM shows that a more densely packed structure is formed during consolidation, suggesting that shear strength and stiffness of agarose gels will increase, which is similar to the behaviour of most soils. The consolidation behaviour of Agarose LM shows a large proportion of

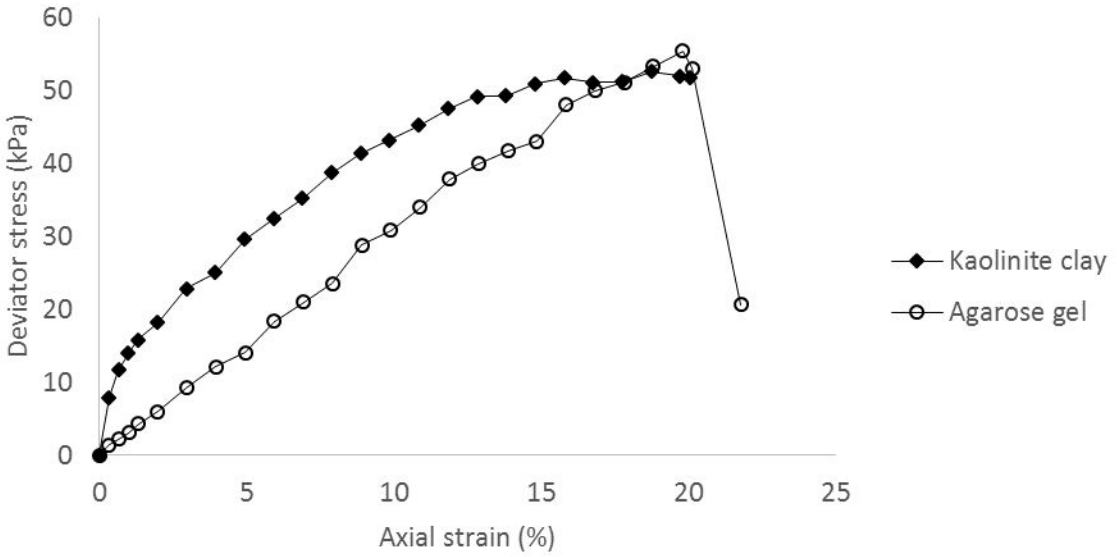
355 secondary compression however this is comparable to the behaviour of normally consolidated clays
356 (Knappett and Craig, 2012).
357
358 Fig. 9.Comparison of permeability Agarose LM gel and soils, adapted from Carter and Bentley (1990).



359
360 However, there are also significant differences between the properties of Agarose LM gel and
361 soils. Agarose LM gel has a higher water content than any soil and consequently a much larger
362 coefficient of volume compressibility than most soils. Only sensitive or highly organic clays and peats
363 exhibit similar values (Carter and Bentley 1990). This high compressibility will result in much higher
364 settlements when loaded, compared to soils.
365 The stress-strain behaviour of Agarose LM is also quite different to that of soil, as shown in
366 Fig. 3 and in Fig. 10, compared to a kaolinite clay. The relationship is linear until failure (about 18-19%
367 axial strain) for Agarose LM gel, in contrast to the behaviour of the kaolinite clay which is only linear
368 at small-strains. For this region of linear stress-strain behaviour, the elastic modulus of very soft clays
369 ranges between 2 and 15 MPa (Bowles 1996) These values are significantly higher than those of
370 Agarose LM gel (approximately 0.3 MPa) but the gel is elastic over a much higher range of strains and
371 shows significant recovery on unloading (Fig. 5). The implications of this are that Agarose LM gel may
372 not be suitable as a soil analogue for an application where settlements must be reproduced. Agarose

LM gel will experience higher short-term settlements and less plastic deformation than a fine-grained soil.

Fig. 10. Comparison of stress-strain relationship of Agarose LM gel and kaolinite clay.



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Conclusions

A programme of geotechnical tests and SEM was performed in order to determine the physical and mechanical properties of Agarose LM gel relevant to soil behaviour. A single concentration of agarose (6%m/v) was used for all mechanical tests. It should be noted that other types of agarose, or other concentrations may behave differently.

It is found that although the gel has a fibrous rather than granular structure, it has similar properties to some saturated cohesive soils such as soft clays or peats, including pore size, undrained strength, permeability and coefficient of consolidation. The comparable pore size to these types of soil means that bacteria are likely to grow and migrate in a similar way in both materials, making 6% Agarose LM gel a reasonable analogue if these aspects are of interest. Similarly, the comparable permeability to saturated cohesive soils means that the gel is a suitable analogue for developing bio-

391 mediated methods of contaminant removal, or for passive soil cementation methods which depend
392 on the flow of water through soils. Agarose LM gel is also currently being used as a soil analogue for
393 the development of a pressure-sensitive biological cementation method, as described by Dade-
394 Robertson *et al* (2018). This requires similar long-term behaviour of a saturated soil under load, so the
395 consolidation behaviour of Agarose LM gel makes it an ideal analogue in this case.

396 However, Agarose LM gel displays a much higher compressibility than soils and also behaves
397 mainly elastically, with very little permanent strains developing when unloaded. This means that
398 Agarose LM gel may not be a suitable soil analogue for applications where amount of settlement,
399 especially short-term settlement is important.

400 Agarose LM gel provides excellent conditions for growing and monitoring bacteria with a high
401 degree of chemical and physical control and many of the physical and mechanical properties of
402 cohesive soil are reproduced. Therefore Agarose LM gels can be an attractive option as a soil analogue
403 for researchers working in a laboratory environment on prototype advanced bio-mediated ground
404 improvement methods for these types of soils, for example contamination remediation and passive
405 or pressure responsive bio-cementation. Further investigation will be required to determine the
406 effects of different solvents on the mechanical properties of the gel, as well as different concentrations
407 and types of agarose. The range of properties available by changing these parameters may widen the
408 potential uses of agarose gel as a soil analogue.

409

410

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Tables

Table 1. Porosity, void ratio and moisture content.

	Porosity, *n*	Void ratio, *e*	Moisture Content, *w*
Theoretical	0.94	16.1	16.7
Average Experimental	0.96	26.0	15.8

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Table 2. Shear strength, maximum axial strain and elastic modulus**.**

Confining pressure (kPa)	Undrained Shear strength, c_u (kPa)	Maximum strain (%)	Elastic modulus (kPa)
100	23	18	268
200	25	18	297
300	28	19	299
400	28	18	328
500	31	19	353

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Table 3. Coefficient of consolidation, c_v for each consolidation stage.

Axial Stress (kPa)	Coefficient of Consolidation, c_v (m^2/year)
3	3.13
6	0.89
12	1.16
25	1.56
50	3.87

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Table 4. Coefficient of volume compressibility, m_v for each consolidation stage.

Axial Stress (kPa)	Coefficient of Volume Compressibility, m_v (m^2/MN)
3	3.26
6	0.59
12	0.56
25	0.3
50	0.3

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Table 5. Derivation and values of vertical permeability, k_v.

Pressure gradient (kPa)	Flow rate (mL/min)	Specimen length (mm)	Area (mm^2)	Vertical permeability, k_v (m/s)
25	0.000088	74.35	1046.35	$4.1x10^{-11}$
50	0.00038	75.40	1046.35	$8.8x10^{-11}$
100	0.00041	74.96	1081.03	$4.6x10^{-11}$
150	0.00045	75.60	1086.86	$3.4x10^{-11}$

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Table 6. Comparison of mechanical properties Agarose LM gel and soils.

Properties	Values	Comparable to
Water content (%)	1583	-
Porosity	0.96	-
Void ratio	26.0	-
Pore size (μm)	0.21 – 0.39	Homogeneous clay soils (0.2-1)[1]
Undrained shear strength, c_u (kPa)	27	Soft clays (20 - 40kN/m^2)[2]. Kaolinite clay (c_u = 25-27kPa)[3]
Maximum axial strain (%)	18	Kaolinite clay (20%)[3]
Elastic modulus (kPa)	309	-
Coefficient of consolidation (m^2/year)	0.89 - 3.87	Organic silts (0.6 - 3m^2/year), glacial clays (2.0 – 2.7m^2/year) and Chicago silty clays (2.7m^2/year)[2]
Coefficient of volume compressibility (m^2/MN)	0.3 - 3.27	Sensitive clays (0.3 – 1.5m^2/MN) and highly organic soils (1.5m^2/MN)[2]
Vertical permeability (m/s)	3.4×10^{-11} – 8.8×10^{-11}	Homogeneous clay soils (see Fig. 9)

[1](Powrie 2014)
[2](Carter and Bentley 1990)
[3]Experimental testing

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Figure Captions

Fig. 1. SEM images of microstructure at mass concentrations of 2% (left), 4% (middle) and 6% (right).

Fig. 2. Pore size as a function of agarose concentration.

Fig. 3. Stress-strain relationship at different confining pressures, with inset of samples showing failure surface.

Fig. 4. Unconsolidated-undrained triaxial test results in the form of Mohr's circles.

Fig. 5. Loading and unloading behaviour at different strain levels.

Fig. 6. Anisotropic consolidation behaviour at different stress levels.

Fig. 7. Derivation of t_{90}.

Fig. 8. Microstructure of consolidated (right) and unconsolidated (left) Agarose LM gel.

Fig. 9. Comparison of permeability Agarose LM gel and soils, adapted from Carter and Bentley (1990).

Fig. 10. Comparison of stress-strain relationship of Agarose LM gel and kaolinite clay.

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