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INTRUSION OF POLYETHYLENE GLYCOL DURING OSMOTIC TESTS: IDENTIFYING CELLULOSE ACETATE DEGRADING MICROBES

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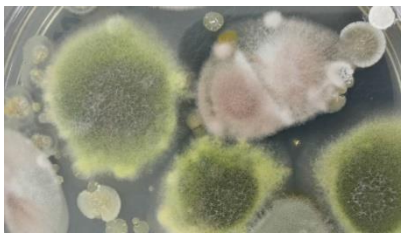
Graphical abstract



Abstract

Cellulose acetate semipermeable membranes and polyethylene glycol (PEG) solutions are commonly used to apply suction in soils using the osmotic technique. The structural integrity of the membrane is crucial to maintain a consistent suction value throughout a test. The membrane however, is vulnerable to microbial attack, which in turn could lead to intrusion of PEG into soil specimens. In this study, osmotic test was carried out on initially saturated Andrassy bentonite specimen. PEG 6000 and membrane with molecular weight cut-off (MWCO) value of 3500 was used to apply suction of 3.4 MPa. Soil specimen and PEG solution after the osmotic test were examined for the presence of any potential cellulose or acetate degrading microbes. Test results indicated that both cellulose degrading bacteria and fungi were present in the PEG solutions. Addition of penicillin was found to be less effective in removing these microbes. However, 70% ethanol may be used to prevent cross contamination during handling of specimens. It is anticipated that eliminating these microbes is crucial to prevent intrusion of PEG in osmotic tests.

Keywords: clay, microbes, suction, osmotic, cellulose, PEG

1.0 INTRODUCTION

Soil is considered the most complex ecology that comprises by an immense variety of microbes which includes both bacteria and fungi [1]. Soil microbes are essential in the degradation and decomposition of organic matter within soil. In recent years, the importance of soil microbes have been recognized. As more knowledge is accumulated through research findings and technology development, a new branch of geotechnical engineering namely, microbial geotechnology has been introduced [2]. The study of soil microbes has been of interest of geotechnical engineering, as the elements contained within soil can greatly influence the composition of microbial community, which in turn can affect the soil properties and engineering behavior [3].

The engineering behavior of unsaturated soils (viz. shear strength, volume change, permeability) due to changes in the water content are commonly predicted by establishing the suction-water content soil-water characteristic curves (SWCCs) [4]. Generally, the suction-water content SWCCs are established using various laboratory techniques [5]. Recently, osmotic technique has gained widespread acceptance as a reliable method for controlling suction in soil specimen [6]. In this technique, a soil

specimen is brought in contact with a solution of polyethylene glycol (PEG) of a pre-determined concentration separated by a semipermeable membrane. Several researches have used the osmotic technique to study the water retention behavior of soils. In addition, the technique has also been used to study the volume change behavior of soils as affected by changes in the soil suction.

Cellulose acetate membranes are generally used in osmotic tests. Cellulose acetate is an acetate ester of cellulose having the chemical formula: $C_6H_7O_2(OH)_3$ [7]. The main advantage of osmotic technique is that with reasonable combination of different weight cut-off (MWCO) semipermeable membrane and different molecular weight PEG having varying concentration can be used to apply different suction. In the event of equalisation of the osmotic suction on either side of the semipermeable membrane, ions are expelled out of the clay-water system and the technique controls matric suction. Literature suggested that, the osmotic technique has been successfully been used for applying suctions up to 1.5 MPa. Although the technique can be further extended to 12 MPa using smaller molecular weight PEGs (i.e. PEG 1500)[8], the application of osmotic technique at higher applied suction appears to be limited. This could be due to semipermeable having smaller MWCO are not readily available.

The main limitation of osmotic technique is associated with the intrusion of PEG into soil specimens [9]. It has been hypothesized in the past that the intrusion of PEG occurs either due to failure of the semipermeable membrane in restricting the passage of PEG molecules or a degradation of PEG molecules into smaller sizes. Tripathy et al [10] noted that, intrusion of PEG occurs due to significant alterations in the pore size of semipermeable membrane after osmotic test at higher applied suction. Alteration in the pore size may enabled the passing of PEG molecules into soil specimens. The magnitude of alteration was found to be significant at higher applied suction using PEG 6000 along with MWCO 3500 membrane.

Slatter *et al*. [11] and Monroy *et al*. [12] stated that cellulose acetate membranes are susceptible to bacteria attacks which may lead to the alteration of the pore size. These bacteria could have originated from the soil specimen. During equalization on either side of the permeable membrane, it is expected that microbes from the soil-water mixture within the membrane are expelled out along with water and ions into PEG solution. Interestingly, [13][14] showed that PEG has antibacterial properties. However, the use of PEG solution alone is not sufficient as the problem with intrusion of PEG into soil specimen still prevails. The antibacterial properties of PEG is ineffective against some bacteria. Some strain of bacteria utilizes PEG as carbon source (i.e. substrate) and can grow in PEG solution [15][16][17]. With regards to PEG degradation, previous study conducted on PEG solutions before and after osmotic test revealed that no changes were observed on the Fourier Transform Infrared (FTiR) spectrum, indicating no degradation occurred to the PEG molecules throughout a testing period of 15 days [10]. However, it cannot be ruled out that, these bacteria may also degrade hydrated PEG molecules into smaller molecules which leads to more intrusion of PEG into soil specimens. In order to prevent bacteria attack on the membrane and PEG molecules, Kassif and Ben Shalom [18] suggested that penicillin is added into PEG solutions prior to osmotic test. Penicillin is an antibiotic derived from certain strains of fungi. It has been shown to be effective in removing various type of bacteria [19].

In addition to bacteria attack, studies have shown that fungi also has the ability to degrade cellulose or acetate. Thus, it is anticipated that the structural integrity of the membrane could also be affected by the presence of fungi. The characterization of soil microbes is an important factor to take into consideration as there have been numerous works stating that most soil fungi and some bacteria are able to degrade cellulose [20][21][22] and acetate [23]. Therefore, the presence of these microbes have the potential to breakdown the cellulose acetate membrane bounding the soil specimen, thus causing PEG to intrude during the osmotic test.

In this study, the potential cellulose acetate degrading microbes present in the PEG solution before and after the osmotic test is determined. In addition, the effectiveness of penicillin in removing these microbes during osmotic test was also evaluated.

2.0 EXPERIMENTAL

2.1 Determination of geotechnical and microbiological properties of bentonite used

The physical and microbiological properties of the Andrassy bentonite was first determined following standard laboratory procedures. The water content, specific gravity, liquid and plastic limits were determined following BS 1377:1990. The shrinkage limit of the clay was determined following ASTM D4943:2008. Both the specific surface area and cation exchange capacity were determined following ethylene glycol monoethyl ether (EGME) [24] and ammonium acetate method [25], respectively.

The microbiological properties of the bentonite, namely bacteria and fungus determination were carried out following plating, slide culture, streaking and isolation techniques [26]. Potato dextrose agar (PDA) was used for culturing fungi, whereas Nutrient agar (NA) was used to culture bacteria. The clay specimen was initially suspended in 0.9% NaCl solution to separate the microbes from the soil [27].

Identification of the specific strain of each microbes after isolation was carried out in an independent laboratory using polymerase chain reaction (PCR) protocol and referred to international microbiological characterization database.

2.2 Osmotic tests

The osmotic tests were carried out on an initially saturated Andrassy bentonite specimen. The tests were carried out following the experimental method suggested by Delage et al. [28]. Bentonite-water mixtures were prepared at a targeted water content equal to about 1.2 times liquid limit of the bentonite. One suction level was considered. PEG 6000 was used along with Spectra/Por MWCO 3500 membrane for applying suction of 3.4 MPa. Deionized water was used for preparing the bentonite-water mixture and the PEG solution. The suction of the PEG solution was measured using WP4C chilled-mirror dew-point hygrometer following [8]. The semipermeable membranes were immersed in deionized water for approximately 30 min to remove glycerin preservative coating prior tests. The tests were carried out for a period of 7 days. In addition, a separate osmotic test was conducted by adding few drops of penicillin in the PEG solution to investigate the effectiveness of penicillin in removing any presence of microbes that can potentially degrade the membrane.

2.3 Determination microbes in PEG solution

The PEG solution before and after the osmotic test were considered for identification of microbes present. Approximately 10 ml of PEG solutions were pipetted from the bulk solution for the determination of microbes' availability.

3.0 RESULTS AND DISCUSSION

3.1 Geotechnical and microbiological properties of soil

The properties of Andrassy bentonite is presented in Table 1. The bentonite in this study was found to exhibit large surface area and high surface charge characteristics which makes it ideal for soil microbes [29]. Referring to Table 1 it was found out that three microbes were present within the soil. All three microbes are considered to be common soil microbes. Interestingly, two strains of fungi (i.e. *Paecilomyces lilacinus and Trichoderma atroviridae)* found in the soil specimen has the potential to degrade cellulose [30][31].

The final water content of the soil specimen after osmotic test was found to be 52.58%. No significant differences were noted between the water contents of soil specimens tested with and without the addition of penicillin (i.e. ± 3% variation). Some clear residue was observed on the surface of the soil. This clear residue changed to white patches after allowed to dry in an oven, indicating that intrusion of PEG had occurred. Similar observation was made by [32] on different type of bentonite specimen.

Table 1 Geotechnical and microbiological properties of Andrassy bentonite

Geotechnical properties	
Specific gravity, G_s	2.78
Liquid limit, w_l (%)	129.30
Plastic limit, w_p (%)	46.12
Shrinkage limit, w_s (%)	34.00
Specific surface area, S (m^2/g)	734.27
Cation exchange capacity, B (meq/100g)	42.77
Microbial properties	
Bacteria	*Bacillus anthracis*
Fungus	*Paecilomyces lilacinus* *Trichoderma atroviridae*

Test results for both NA and PDA plating tests after osmotic tests are shown in Figs. 1 and 2. Significant differences were observed between plates obtained for determination of microbes in soil specimen and plates obtained from PEG solutions after osmotic tests, indicating that different strains of microbes were present. Bacteria colonies were abundant and appeared to overlap to each other as compared to their fungi counterparts. Each stain were carefully isolated and characterised. Comparison of plates obtained from PEG solution with and without the addition of penicillin shows no significant difference in both NA and PDA plates.

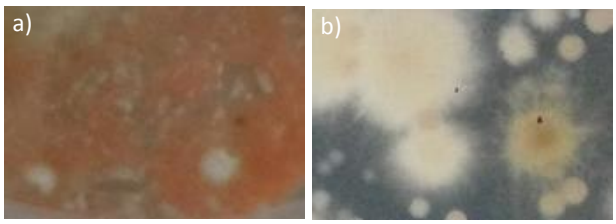


Figure 1 PEG solution after osmotic test (a) NA plate and (b) PDA plate

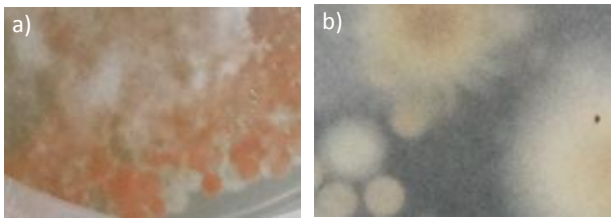


Figure 2 PEG solution after osmotic test with addition of penicillin (a) NA plate and (b) PDA plate

Identification of each plates revealed that at the end of the osmotic test, additional strain of microbes existed (see Table 2). Four additional bacteria and two fungi colonies were successfully identified. It was believed that these additional strains were introduced to the PEG solution during sample preparation and handling of the specimen throughout the commencement of the osmotic test.

Table 2 Types of microbes present in PEG solution after osmotic tests

After osmotic test	After Osmotic Test + Penicillin
Bacteria	**Bacteria**
Bacillus anthracis	*Bacillus anthracis*
Staphylococcus aureus	*Staphylococcus aureus*
Micrococcus luteus.	*Micrococcus luteus.*
Achromobacter xylosoxidans	*Achromobacter xylosoxidans*
Escherichia coli	*Escherichia coli*
Fungus	**Fungus**
Paecilomyces lilacinus	*Paecilomyces lilacinus*
Trichoderma atroviridae	*Trichoderma atroviridae*
Fusarium proliferatum	*Fusarium proliferatum*
Rhodotorula mucilaginosa	*Rhodotorula mucilaginosa*

Previous studies by [33][34] proved that these strains (i.e. *Staphylococcus sp.*, *Micrococcus sp.*, and *Escherichia coli*) can originate from human interactions. Based on the test results, there was no reduction in the types of microbes and colonies found in the PEG solutions with penicillin as compared to PEG solution without penicillin. Studies by [35][36] have shown that penicillin is ineffective against fungi and is only effective against certain bacterial colonies. Thus, for PDA plates (Fig. 1b and Fig. 2b), it is not surprising to see that fungi persisted and remained unchanged. Although the PEG solutions were contaminated with additional strains of fungi (i.e. *Fusarium proliferatum* and *Rhodotorula mucilaginosa*), there has been no evidence that these fungi have the potential of degrading cellulose or acetate based material.

In the case of NA plates, it was found out that all the strains were somewhat immune to penicillin. Penicillin was unable to eliminate any of the microbes. This may be attributed to the development of antibiotic resistance towards penicillin [34][37][38]. In order to remove contamination of microbes from external sources (i.e. due to handling) an attempt was made to incorporate the use of 70% ethanol spray during preparation of soil specimens prior to osmotic test. Ethanol are extensively been used for disinfection and elimination of microbes due to cross contamination in microbiological applications [26]. Figure 3 shows the NA and PDA plates obtained after osmotic test with addition of penicillin and the use of 70% ethanol. NA plates shows that the orange colonies were no longer visible from the PEG solution obtained after the osmotic test.

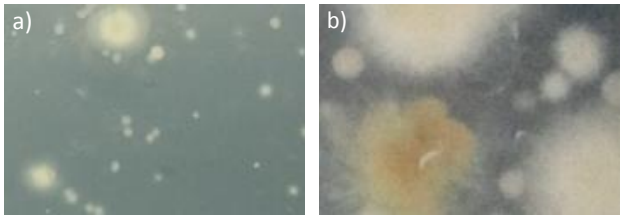


Figure 3 PEG solution after osmotic test with addition of penicillin and the use of 70% ethanol (a) NA plate and (b) PDA plate

For PDA plates however, no significant difference were noted (see Fig. 3b). Identification of NA plates revealed that some reduction in the types of bacteria occurred. Three bacteria strains, namely *Micrococcus luteus, Achromobacter xylosoxidans* and *Escherichia coli* were removed, whereas, for PDA, all fungi strains remained unaffected. The usage of ethanol prior to handling of specimen can eliminate some bacterial strains. This can be seen in the elimination of certain strains such as *Micrococcus luteus, Achromobacter xylosoxidans* and *Escherichia coli*, which is susceptible to ethanol's antimicrobial properties [39][40][41]. The microbes present in the soil however, were found to be unaffected, as initially, ethanol was not mixed with the soil. In addition, it is anticipated that the effectiveness of ethanol would also decreased when the soil specimen was submerged inside the PEG solution during the test. The addition of penicillin and the use of 70% ethanol were found to be less effective in removing fungi found in this study. Furthermore, due to strict requirements, penicillin is not readily available and prescriptions from qualified medical personnel are often required.

In the past, much focus have been given to degradation of semipermeable membrane due to bacterial attack [5][10][18]. However, the degradation ability of cellulose acetate is not exclusive to bacteria only [30][31]. The presence of cellulose degrading fungi such as *Paecilomyces lilacinus* and *Trichoderma atroviridae* within soil specimens and PEG solutions may inevitably affect the structural integrity of the membrane and in turn caused the intrusion of PEG molecules into soil specimens to occur.

4.0 CONCLUSION

Detailed laboratory investigations were carried out to determine the presence of cellulose acetate degrading microbes in osmotic test. Osmotic tests were carried out on initially saturated slurried Andrassy bentonite specimens using PEG 6000 and cellulose acetate MWCO 3500 semipermeable membrane. Osmotic tests were carried out with and without the

addition of penicillin. Based on the findings of this study, the following conclusion can be drawn:

1. Both bacteria and fungi were present in soil as well as in the PEG solutions after osmotic tests. Potential fungi were identified as possible microbes that may contributed to the intrusion of PEG molecules into soil specimens.
2. Penicillin is ineffective in removing fungi from soil as well as PEG solution. Thus, presence of any cellulose degrading fungi may cause deterioration of the membrane pore size.
3. Cross-contamination during handling occurred and additional microbes were observed.
4. The use of 70% ethanol prior to preparing soil specimen assisted in minimising bacterial cross-contamination during handing of specimen.

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