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AN EVALUATION OF OSMOTIC TECHNIQUE UNDER ULTRAVIOLET GERMICIDAL IRRADIATION EXPOSURE

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ABSTRACT: The osmotic technique, which uses polyethylene glycol (PEG) solutions of varying concentrations with semipermeable membranes of different molecular weight cutoffs (MWCO), is commonly used to apply suction in soils. Cellulose acetate membranes which are most commonly used, are susceptible to microbial attacks. This in turn will lead to the intrusion of PEG into soil specimens. Osmotic and vapour equilibrium techniques are often used to establish drying suction-water content soil-water characteristic curves (SWCC). In this study, suctions of 0.11 to 300 MPa were applied on Andrassy bentonite slurries. At higher applied suctions, the osmotic tests were carried under short length ultraviolet germicidal irradiation (UVGI). In addition, Atomic Force Microscopy (AFM) and Fourier Infrared (FTiR) were employed to evaluate the changes in the semipermeable membranes and PEG molecules, respectively. The water content of the clay obtained from the osmotic tests was found to be greater at the overlapping suction region. Interestingly, under UVGI exposure, the water content was found to be in good agreement with the water content determined using the osmotic technique at low suctions and the vapour equilibrium technique at higher suctions. FTiR spectrum and AFM results revealed that some changes had occurred on both the PEG and in the membrane pore sizes. However, these changes did not affect the final water content in the bentonite and therefore, more precise suction-water content SWCC for the clay could be established.

Keywords: Clay, Suction, Osmotic, Microbes, PEG, UV, Cellulose Acetate

1. INTRODUCTION

Bentonite is expansively used in various geo-environmental applications. Bentonite in various forms such as loose powder, compacted and slurry have been used in the construction of liners, engineered barriers, isolation walls and backfill material [1][2]. Bentonites have been considered due to their unique containment properties (i.e. low permeability, high sorption capacity, self-sealing membrane characteristics, and durability in a natural environment) [3][4]. As of late, the study of soil microbes and their interactions in bentonite buffer has been of interest in nuclear waste repository applications [5]. Previous studies have shown that the presence of microbes can affect the geochemistry of bentonites and thus, influence their properties and engineering behavior [6][7][8][5].

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). In the laboratory, the suction-water content SWCCs are commonly established using various techniques to predict the changes in the water content in soils [9][10]. Recently, the vapour equilibrium and osmotic techniques have gained widespread acceptance as reliable methods for controlling suction in soil specimens [11]. The vapour equilibrium technique, which utilizes thermodynamic principles to control the relative humidity of various acid and salt solution vapours in a closed system, is used to apply suction in soil [9]. In the osmotic technique, on the other hand, the soil specimen is placed in semipermeable membrane and is brought into contact with polyethylene glycol (PEG) of predetermined concentration. In this technique, two polymers are commonly used, namely aqueous solution of (PEG) and cellulose acetate semipermeable membrane [12].

One of the main drawbacks of the osmotic technique when using PEG 6000 along with molecular weight cut-off (MWCO) 3500 semipermeable membrane, is the intrusion of PEG solution into the soil specimen particularly at higher applied suction [13]. This problem occurs due to the breakage of the semipermeable membrane by soil fungi [14]. As soil hosts a diversity of microbes, the breakage of the semipermeable membrane in osmotic tests is found to be inevitable. Removal of soil microbes prior to conducting osmotic tests is therefore crucial to ensure precise determination of soil-water SWCCs.

The main focus of this paper is to evaluate the use of short-length ultraviolet germicidal

irradiation (UVGI) to remove soil microbes prior to conducting osmotic tests. In this study, the suction-water content SWCC for Andrassy bentonite was established using both osmotic and vapour equilibrium techniques. The microbial characteristics of the bentonite were established before the removal of said microbes by exposing both PEG solutions and the soil specimens to UVGI. Atomic Force Microscopy (AFM) and Fourier Transform Infra-red (FTiR) spectroscopy were employed before and after exposure of UVGI. to determine if there any changes to PEG molecules and cellulose semipermeable membrane as a result of the irradiation.

2. LITERATURE REVIEW

2.1 Suction-Water Content SWCC

PEG is a hydrophilic polymer made up of molecular chains having the chemical formula: $HO\text{-}[CH_2\text{-}CH_2\text{-}O]_n\text{-}H$ [9]. The use of PEG–water mixtures along with suitable semipermeable membranes enables the application of osmotic gradients in an osmotic system. During the commencement of an osmotic test, a soil specimen is enveloped by a semipermeable membrane and immersed in an aqueous solution of PEG of varying concentrations. Cellulose acetate, an acetate ester of cellulose ($C_6H_7O_2(OH)_3$) is commonly used for this purpose [14]. In some cases, polysulfonate membrane is also used [9]. The semipermeable membrane is expected to prevent the passage of PEG molecule to soil–water systems. A review of literature suggested that, the osmotic technique has been successfully used for applying suctions in soils up to 1.5 MPa. Although the technique can be further extended to 12 MPa using smaller molecular weight PEGs (i.e. PEG 1500) [12], the application of osmotic technique at higher applied suction appears to be limited to PEG 6000 only. This could be due to unavailability of lower MWCO semipermeable membranes [14]. For suctions higher than 10 MPa, the vapour equilibrium technique is often opted for. The vapour equilibrium technique has been used to apply total suction from 3 up to 1000 MPa [9][11].

In order to obtain a continuous suction-water content SWCC that covers a wide range of suction for any given soil, suction-water content from both techniques are commonly plotted together. Studiesin the past have shown disagreement between the test results from both vapour equilibrium and osmotic tests at higher applied suctions [15][10]. The differences were mainly attributed to alterations of the membrane pore sizes which enabled the crossing of PEG molecules into soil specimens [13]. The magnitude of the alteration was found to be significant for lower MWCO membranes.

2.2 Intrusion of PEG

Soil microbes (i.e. bacteria and fungi) exist in both naturally occurring and commercially available bentonites [8][5]. The biodegradation of cellulose acetate based material by soil microbes has been studied for a long time by number of authors [16][17]. It is anticipated that, these microbes were responsible for the degradation of the membrane. The antibacterial property of PEG was found to be insufficient in removing soil microbes [14]. In geotechnical application, Kassif & Ben Shalom [18], recommended the usage of penicillin during laboratory tests to eliminate the bacteria to ensure long-term performance of cellulose acetate membranes. However, a recent study revealed that the breakage of the membrane is due to biodregadation of cellulose acetate by soil fungi [14]. Mohd Tadza et al. [14] also noted that the use of penicillin is ineffective in removing soil fungi. Thus, other methods of disinfection may be required to eliminate the presence of cellulose degrading fungi in soil specimens.

2.3 Ultra Violet Germicidal Irradiation

The application of short-length UVGI in disinfection and removal of microbes is widely used and accepted in the microbiology community [19]. This method was also applied in disinfection of medical instruments and operation theaters [20]. The UV exposure at 200-280 nm for a certain period of time damages cell's DNA and RNA, thus destroying the ability of the microbes to reproduce [21]. The germicidal effectiveness peaks at UVC, which is the subdivision of the UV wavelength (between 260-265 nm) [22]. Various types of soil bacteria and fungi were completely eliminated when exposed directly to UV lamp under laboratory conditions [23][21].

The main concern of using UVGI in osmotic technique is that the UV exposure may also lead to degradation of both the PEG molecules and cellulose acetate membrane. PEG can be photochemically degraded when exposed to UV. However, prolonged exposure is required. Das & Gupta (2005) noted that some changes in the FTiR spectrum of PEG when exposed under UV lamp for more than 1 hour. In the presence of oxidation chemicals such as hydrogen peroxide and titanium oxide, the degradation of PEG in aqueous form was found to occur at shorter periods of exposure

[25][26].

Direct degradation of cellulose acetate under UVGI has not been reported elsewhere. However, previous researches have shown that, cellulose acetate based materials were found to degrade underUV exposure at different wavelengths (i.e. 275-390 nm) in the presence of photo sensitizers and oxidation catalysts [17]. Thus, the objective of this study is to explore the effect of UVGI exposure within this context.

3. EXPERIMENTAL TECHNIQUES

3.1 Determination of Geotechnical and Microbiological Properties of Bentonite Samples

The physical and microbiological properties of the Andrassy bentonite was first determined following standard laboratory procedures described in [10][14]. The microbiological properties of the bentonite, namely bacteria and fungi determination were carried out following plating, slide culture, streaking and isolation techniques [19]. 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]. Isolation of each microbe was carried out in an independent laboratory using the polymerase chain reaction (PCR) protocol and identification of the specific strain was done by referring to the international microbiological characterization database. and no vertical lines or borders are needed.

3.2 Establishing the drying suction-water content SWCC

Bentonite slurry was initially prepared by mixing bentonite powder thoroughly with deionized water to slightly greater than liquid limit (i.e. 1.2 x LL). The bentonite–water mixture was stored in sealed plastic bags and kept in an air-tight container to allow for water equilibration to take place for about 7 days prior to preparing the specimens for the laboratory tests.

Osmotic tests were carried out by applying suctions of 0.15, 0.25, 0.42, 1.03, 1.7, 3.65, 5.08, 6.64, 7.74, and 9.96 MPa using two types of PEG. PEG 20 000 was used for applying lower suctions up to 2.67 MPa, whereas PEG 6000 was used for applying suctions greater than 2.67 MPa. The applied suctions in vapor equilibrium were 3.6, 14.58, 23.58, 39.38, 111.77 and 262.75 MPa corresponding to the relative humidities (RHs) of saturated solutions of K_2SO_4, KNO_3, KCl, NaCl, K_2CO_3, and LiCl, respectively. The suctions of each PEG and saturated salt solutions were measured using a chilled mirror dew-point hygrometer. Decagon WP4C was used for this purpose.

PEG 20 000 and Spectra/Por semipermeable membranes with MWCO value of 14 000 were used for applying suctions lower than 3.0 MPa, whereas PEG 6000 and Spectra/Por membranes with MWCO value of 3500 were used for applying higher suctions. Specimens for the osmotic tests were prepared using the bentonite slurry in cellulotic tubes of the semipermeable membranes (MWCO 14 000 and MWCO 3500). Both ends of the semipermeable membrane tubes were then securely fastened using polypropylene clips prior to immersing the specimens in PEG solutions.

For applied suction higher than 3.0 MPa using PEG 6000, duplicate tests were conducted under UVGI exposure. For this purpose, the tests were conducted using Esco Airstream Horizontal Laminar Flow Clean Bench (Glass Side Wall). The UV lamp automatically switched off after 10 minutes. For establishing suction-water content relationship using vapor equilibrium, bentonite slurries were initially prepared in 38 mm diameter stainless steel rings. Once prepared, the slurry specimens were carefully extruded and transferred directly into test desiccators.

3.3 AFM Analyses

After completion of the osmotic tests, the membranes were carefully removed and rinsed before being directly transferred to a Nanowizard II scanning probe AFM from JPK Instruments for morphological scanning.

3.4 FTiR Analyses

A PerkinElmer Spectrum 100 spectrometer was used for studying the chemical and molecular changes of the PEG solutions. FTIR spectroscopy generates spectrum patterns according to the infrared intensity (i.e. wavenumber or wavelength) and the fraction of infrared transmitted by certain chemical bonds [28].

The spectrum patterns were then interpreted following methods described by Morrison & Boyd [29]. A sudden shift or distortion to the spectrum will generally indicate degradation or changes to either chemical bonds or molecule structure [20]. PEG 6000 solutions at the end of osmotic tests (i.e. with and without UVGI exposure) were collected and tested.

4. RESULTS AND DISCUSSION

4.1 Geotechnical and Microbiological Properties of Andrassy Bentonite

The properties of Andrassy bentonite is presented in Table 1. The bentonite in this study was found to exhibit a large surface area and high surface charge characteristics which make it ideal for soil microbes [34]. Referring to Table 1, it was found out that eight microbes were present within the soil, (i.e. Bacteria: *Bacillus anthracist, Staphylococcus aureus, Micrococcus luteus, Achromobacter Xylosoxidans; Fungi: Paecilomyces lilacinus, Trichoderma atroviridae, Fusarium proliferatum, Rhodotorula mucilaginosa).*

All eight 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 [14]. Remarkably, no presence of microbes were observed on both PDA and NA plates after UVGI exposure, indicating that complete removal of microbes were obtained after 10 minutes of UVGI exposure.

Table 1 Geotechnical properties of Andrassy bentonite

Geotechnical properties	
Specific gravity, Gs	2.78
Liquid limit, *wl* (%)	129.30
Plastic limit, *wp* (%)	46.12
Shrinkage limit, *ws* (%)	34.00
Specific surface area, *S* (m_2/g)	734.27
Cation exchange capacity, B (meq/100g)	42.77

4.2 Effect of UVGI on Suction Calibration Curve

Figure 1 shows the measured concentrations of PEG solutions with and without UVGI exposure used in this study. Similarly, the calibration curves established using Eq. 1 [12] were plotted together for comparison.

$$s = \alpha c^3 + \beta^2 + \gamma c \qquad (1)$$

where *s* is the suction and *c* is the concentration of PEG solution in g of PEG/ g of water. Fitting parameters, α, β and γ are -3.527, 14.334 and -0.8955, respectively. The suctions measured increased with an increase in the concentrations of PEG solutions. Interestingly, good agreement was noted between the suction measured with in this study with the calibration curve provided by Tripathy and Rees [12]. Interestingly, similar suction values were noted for the calibration curve established for PEG 6000 solutions exposed to UVGI. Hence, no recalibration of the suction and concentration curve was required for PEG 6000 exposed to UVGI.

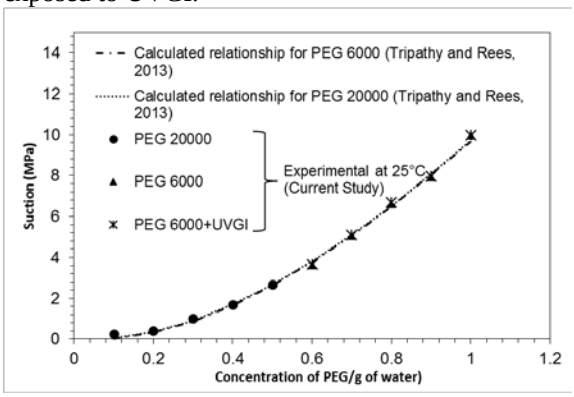

Fig 1 Measured suction versus concentration of PEG/g of water

4.3 Suction-water content SWCC

Figure 2 shows the equilibration plot at applied suction of 3.65 MPa using PEG 6000. Some differences were noted between the equilibration time for specimen with and without UVGI exposure.

The specimen that has undergone UVGI was found to equilibrate much longer (i.e. 8 days) as compared to 6 days for the specimen without UVGI exposure. Interestingly, the final water content attained for the specimen exposed under UVGI was slightly greater compared to the specimen that has not been exposed to UVGI. It is expected that a much faster equilibration was obtained due to alteration of the membrane pore sizes.

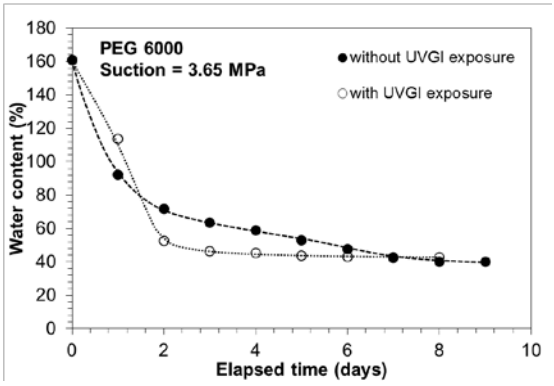

Fig 2 Time equilibration plot in osmotic test at suction 3.65 MPa.

The drying suction-water content SWCC is shown in Fig. 3. The final water contents of the soil specimens were found to decrease with an

increase in the applied suctions. Smooth curve joining the osmotic test at lower suctions (i.e. PEG 20000), and higher suctions (i.e. vapor equilibrium). However, some disagreements were noted between osmotic test results (see PEG 6000 without UVGI exposure) and vapor equilibrium test results. The osmotic tests results at the overlapping suction region were found to remain lower than that of water contents obtained from vapour equilibrium. Similar observation was reported by Tripathy et al. [10] for other types of bentonites. The lower water contents are primarily attributed to the intrusion of PEG solution into the bentonites specimens. On the contrary, water contents obtained using PEG 6000 that has undergone UVGI were found to complement the test results of both osmotic test at lower suctions and vapour equilibrium at higher applied suctions.

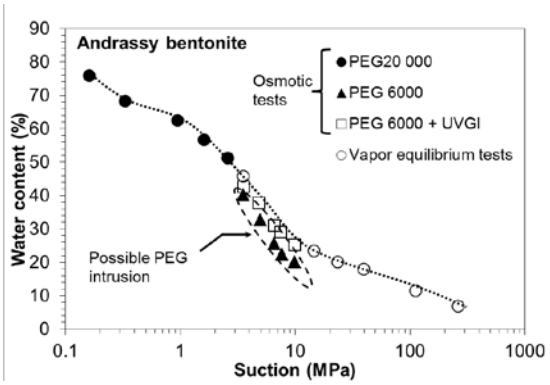

Fig 3 Drying suction-water content SWCC

4.3 Effect of UVGI on Membrane Pore Size

Figure 4a and 4b present typical AFM images of MWCO 3500 membrane with and without UVGI exposure at the end of the osmotic tests. The membrane is composed of series of well-organized regenerated cellulose acetate strands to form a mesh like formation. AFM images revealed that both membrane pore sizes were larger than that specified by the manufacturer. It is believed that these differences were caused by degradation caused by cellulose degrading microbes present within the soil. Similarly, degradation also occurred due to UVGI exposure.

Some differences were noted between the pore size of membrane after the test and the pore size of membrane exposed to UVGI. Differences in the structural arrangements of the strands were also observed. Alteration of the membrane pore sizes by cellulose degrading microbes in this study (i.e. without UVGI) was found to be greater than those that have undergone UVGI exposure. Surprisingly, although alteration of the membrane pore sizes occurred after UVGI exposure, the final water contents determined in the osmotic tests were found to be in better agreement with those of the osmotic test at lower suctions and the vapour equilibrium water contents.

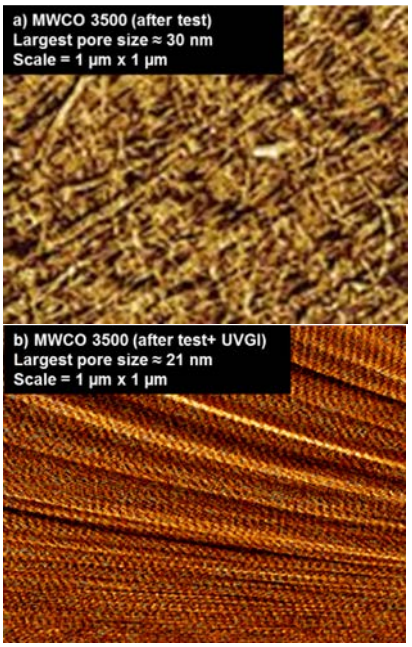

Fig. 4 AFM images of MWCO 3500 semi-permeable membrane after osmotic tests (a) without UVGI and (b) with UVGI exposure

4.4 Effect of UVGI on FTiR Spectrum

Comparison of FTiR spectrums for PEG 6000 solutions after completion of osmotic tests with and without UVGI exposure is shown in Fig. 5. Slight differences were noted on 3369 cm-1 peaks corresponding to O-H bond. It is noted that a slightly higher transmittance were obtained for PEG solution after osmotic tests without UVGI exposure. Apart from that the primary molecular structure of PEG remained somewhat unaffected by the exposure of UVGI, indicating that no degradation occurred.

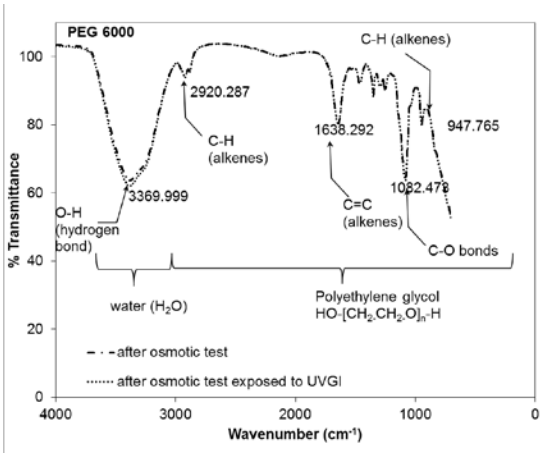

Fig. 5 Comparison of FTiR spectra for PEG 6000 with and without UVGI exposure

5. CONCLUSION

Results of the investigation after conducting osmotic test under UVGI exposure have been presented in this paper. Based on the test results, the following conclusions were drawn:

i. All soil microbes in this study were completely eliminated by 10 minutes of UVGI exposure, and thus degradation of membrane by microbes is prevented.

ii. Water contents obtained after UVGI complemented vapour equilibrium and osmotic tests results at lower suctions, and more precise SWCC were established.

iii. the FTiR Spectrum showed that UVGI had some effects on the O-H bond and pore size of the cellulose acetate semipermeable membrane. These effects were found to be insignificant in the determination of final water contents of the bentonite.

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7. REFERENCES

[1] Shackelford C.D, "Membrane behavior in engineered bentonite-based containment barriers: state of the art". In Proceedings of Coupled Phenomena in Environmental Geotechnics (CPEG 2013), 1–3 July 2013, Torino, Italy, Taylor & Francis Group, London, UK, 2013, pp. 45–60.

[2] Tripathy S., Thomas H., and Bag R, "Geoenvironmental Application of Bentonites in Underground Disposal of Nuclear Waste: Characterization and Laboratory Tests", J. of Hazardous, Toxic, and Radioactive Waste, 2015.

[3] Pusch R. and Yong R.N. Microstructure of Smectite Clays and Engineering Performance. 1st Edition, Taylor and Francis, 2005.

[4] Plotze M., Kahr G., Dohrmann R. and Weber H. P. Hydro-mechanical, geochemical and mineralogical characteristics of the bentonitebuffer in a heater experiment: The HE-B Project at the Mont Terri Rock Laboratory - Elsevier, Physics and Chemistry of the Earth, Vol. 32, 2007, pp. 730-740.

[5] Stone W., Kroukamp O., Moes A., McKelvie J., Korber D.R and Wolfaardt G.M, Measuring microbial metabolism in atypical environments: Bentonite in used nuclear fuel storage, Journal of Microbiological Methods, Vol. 120, 2016, pp. 79-90

[6] Pedersen K., Motamedi M., Karnland O., and Sandén T, Cultivability of microorganisms introduced into compacted bentonite clay buffer under high-level radioactive waste repository conditions, Engineering Geology, Vol. 58, 2000, pp.149–161.

[7] Stroes-Gascoyne S., Hamon C.J., Maak P., and Russell S, The effects of the physical properties of highly compacted smectitic clay (bentonite) on the culturability of indigenous microorganisms, Applied Clay Science, Vol.47, 2010, pp. 155-162.

[8] Lopez-Fernandez M., Fernandez-Sanfrancisco, O., Moreno-Garcia., Martin-Sanchez., Sanchez-Castro I and Merroun M.L, Microbial communities in bentonite formations and their interactions with uranium, Applied Geochemistry, Vol. 49, 2014, pp. 77-86.

[9] Delage, P., Romero, E. and Tarantino, A, Recent developments in the techniques of controlling and measuring suction in unsaturated soils. In Saturated Soils: Advances in Geo-Engineering, Taylor and Francis Group, London, 2008, pp. 33-52.

[10] Tripathy S., M. Tadza M.Y. and Thomas H.R, Soil-water characteristic curves of clays, J. of Canadian Geotechnical Journal, Vol. 51, No. 8, 2014, pp. 869-883.

[11] Blatz J.A., Cui Y. J., and Oldecop L, Vapour equilibrium and osmotic technique for suction control, J. of Geotechnical and Geological Engineering, Vol. 26, No. 6, 2008, pp. 661–673.

[12] Tripathy S. and Rees S.W, Suction of Some Polyethylene Glycols Commonly Used for Unsaturated Soil Testing, Geotechnical Testing Journal, Vol. 36, No. 5, 2013, pp.768-780.

[13] Tripathy S., Tadza M.Y.M. and Thomas H.R, On the intrusion of polyethylene glycol during osmotic tests, Geotechnique Letters, Vol. 1, No. 3, 2011, pp. 47–51

[14] Mohd Tadza M.Y., Farida Asras M.F., Mohd Tadza M.A., Ismail J., Mahazam N. and Mohd Azmi S.M, Intrusion of Polyethylene Glycol During Osmotic Tests: Identifying Cellulose Acetate Degrading Microbes, Jurnal Teknologi (Sciences & Engineering), Vol. 72, 2016, pp. 1-6.

[15] Fleureau J. M., Kheirbek Saoud S., Soemitro R. and Taibi, S, Behavior of clayey soils on drying–wetting paths, J. of Canadian Geotechnical Journal, Vol. 30, No. 2, 1993, pp. 287–296.

[16] Lenz R.W, Biodegradable polymers, Advances in Polymer Science, Vol. 107, 1993, pp. 1-40.

[17] Puls J., Wilson, S.A and Holter. D, Degradation of Cellulose Acetate-Based Materials: A Review, J. Polym. Environment, Vol. 19, 2011, pp. 152-165.
[18] Kassiff G. and Ben Shalom A, Experimental relationship between swell pressure and suction, J. of Géotechnique, Vol. 21, No. 3, 1971, pp. 245–255.
[19] Benson H. J, Microbiological Applications: A Laboratory Manual in General Microbiology. McGraw-Hill Science, 2001.
[20] Katara G, Hemvani M, Chitnis S, Chitnis V, Chitnis D.S, Surface disinfection by exposure to germicidal UV light. Ind J Med Microbiol, Vol, 26, 2008, pp. 241–242.
[21] Kowalski W, Ultraviolet Germicidal Irradiation Handbook, Springer-Verlag Berlin Heidelberg, 2009.
[22] Harm W, Biological Effects of Ultraviolet Radiation, New York: Cambridge University Press, 1980.
[23] Johnson M.L., Berger L., Philips L and Speare R, Fungicidal effects of chemical disinfectants, UV light, desiccation and heat on the amphibian chytrid Batrachochytrium dendrobatidis, Diseases of Aquatic Organisms, Vol. 57, 2003, pp. 255-260.
[24] Das I and Gupta S.K, Polyethylene glycol degradation by UV irradiation, Indian Journal of Chemistry, Vol. 44A, 2005, pp. 1355-1358.
[25] Giroto J.A, Teixeira A.C.S.C, Nascimento C.A.O and Guardani R, Degradation of Poly(ethylene glycol) in Aqueous Solution by Photo-Fenton and H2O2/UV Processes, Ind. Eng. Chem. Res., Vol. 49, No. 7, 2010, pp 3200–3206.
[26] Santos L.C., Schmitt C.C., Poli A.L and Neumann M.G, Photo-Fenton Degradation of Poly(ethyleneglycol), J. Braz. Chem. Soc., Vol. 22, No. 3, 2011, pp. 540-545.
[27] Zborowski M., Malchesky P. S., Jan, T. and Hall G. S, Quantitative separation of bacteria in saline solution using lanthanide Er(II1) and a magnetic field, J. of General Microbiology, Vol. 138, 1992, pp. 63-68.
[28] Roberts J. D. and Caserio, M. C, Basic principles of organic chemistry, California: W. A. Benjamin, Inc., 1965.
[29] Morrison R. T. and Boyd, R. N, Organic chemistry. Allyn and Bacon Inc. 1983.