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The Behaviours of Natural and Sporosarcina Pasteurii (Bacillus Pasteurii) as a Liner

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ABSTRACT: The enhancement of sandy soil as a liner and cover material for usage as waste containment facility was taken into consideration in this work using Bacillus pasteurii to activate the microbial-induced calcite precipitation (MICP) process. This study aimed at determining the physical properties of SPOROSARCINA PASTEURII (BACILLUS PASTEURII) as a liner. The study's sandy soil, which had a natural moisture content of 8.56%, came from Wudil Local Government Area in Kano State, Nigeria. Stepped Bacillus pasteurii suspension densities of 0 cells/ml, 1.5 x 108 cells/ml, 6 x 108 cells/ml, 12 x 108 cells/ml, 18 x 108 cells/ml, and 24 x 108 cells/ml were applied to the sandy soil. The Atterberg's limit test result, which showed that the soils were nonplastic, was used to determine the index properties of the soil. The soil was classified as A-3(0) and SP soil, respectively, according to the AASHTO and USCS classification systems. The specific gravity decreases with increase in *Bacillus pasteurii* suspension density.

KEYWORDS: Sandy soil, Liner, Specific gravity, geotechnics

I. INTRODUCTION

The use of sterile landfills for constricting the movement of leachate of arranged waste into the ground has been a typical practice in created countries. As a result of low penetrability necessity, Compacted Clay Liners (CCLs) has been an adequate procedure for obstruction frameworks to lessen the movement of toxins from the landfill into the ground that contaminate the underground water. Subsequently, the unwavering quality of a landfill is subject significantly on the strength and water powered execution of their obstruction frameworks. Compacted Clay Liners CCL is made in the field established on the dirt's MDD and OMC [1]. The research centre and field examinations announced by [2] recommended that either CCL or Geosynthetic Clay Liner (GCL) lie underneath by a geomembrane can proficiently moderate the shift in weather conditions and dispersion of the contaminations into the ground that dirty drinking water. The impact of a waste disposing of office on groundwater quality use for human utilization vigorously depends on the sort of obstruction which is imagined to limit and control toxin movement [2]. The basic role of designed landfill framework in control offices is to decelerate foreign substance development into the hidden subsurface for the time of both the dynamic removal time and the post conclusion age. Hindrance framework is a significant element of a contemporary designed landfill. The innovation has filled genuinely in the last periods in countries where landfill has been practically speaking. The planned materials utilized for such designs are compacted Clay, geosynthetic Clay liners (GCLs), reused squander, reused squander [3].

II. OVERVIEW

(One of the most important parameters involved in the assessment of engineering facilities for dams and waste containment applications is the hydraulic conductivity of a soil [4]. Due to the importance and sensitivity of hydraulic conductivity in waste containment systems, its values are often given to at least two significant figures rather than being rounded. [5] reported that the MICP approach reduced the hydraulic conductivity by 60–70% for all the sand treated. Researchers from several fields have noted a decrease in the permeability of soils treated with MICP. After treating residual soils, [6] showed a decrease in hydraulic conductivity from 1 × 10−7 to 2.6 ×10−8 m/s. Additionally, depending on the effective size of the soil specimen (D10), typical calcite content, and starting hydraulic conductivity, a drop in hydraulic conductivity of up to four orders of magnitude has been seen. [7]. Additionally, [8] found a maximum reduction of 98.18% in the hydraulic conductivity of compacted lateritic soil treated at 2% water content with a 6.0 × 108 cells/ml suspension density of S. pasteurii. When two different samples of sandy soil were treated with 0.1-1.5% xanthan gum from the bacterial species Xanthomonascampestris, [9] reported a reduction in hydraulic conductivity; the reductions recorded were from 7.16 × 10−3 to 5.75 × 10−5 m/s and from 8.46 × 10−3 to 2.84 × 10−11 m/s for the two samples, respectively. The formation

of biomass or biofilm by some species of the organisms utilized in MICP processes has been observed to be associated with conditions that cause the hydraulic conductivity to decrease, in addition to the blocking of voids in the soil medium by calcite precipitate [10]

III.MATERIAL AND METHODS

MATERIALS

Soil Sample

Sandy soil sample to be used for this research will be obtain from Wudil Local Government Area latitude 1107'86" N and longitude 808'40" E) in Kano State, Nigeria.

Microorganism

The Gram-positive, rod-shaped, spore-forming bacteria found in the sandy soil used in this study is Sporocarcinalpasteurii (S. pasteurii), which was classified according to American Type Culture Collection as ATCC27142. Bacteria were culture in liquid media consisting of 3g Nutrient Broth, 20g of Urea, 10g of NH4Cl, 2.12g NaHCO3 per litre of glass distilled water, with a pH measured at 9.7. Liquid media were sterilized by autoclaving for 20 minutes at 1210C [11, 12]. The cultured S. pasteurii are shown on Plate 1. The role of S. pasteurii was to produce enzyme urease through its metabolic activity under proper cultivation process. The enzyme urease triggered the MICP biochemical reaction by hydrolyzing urea [CO(NH2)2] through the following reaction:

CO(NH2)2+ 3H2O 2NH4+ + HCO3- + OH-

The ammonium (NH4+) increased the pH of the solution and thereby caused the bicarbonate (HCO3-) to precipitate with calcium ion (Ca2+) from the calcium chloride supplied in order to form the calcium calcite (CaCO3-): Ca2- + HCO3------ CaCO3 + H2O

Plate 1: Cultured Bacterial Samples

Cementation Reagents

The raw materials for calcite formation in the MICP process. Is the cementation reagents used in this study comprised 20g urea [CO(NH2)2] and 2.8g of calcium chloride ($CaCl_2$). The cementation reagents also contained 3g nutrient broth, 10g ammonium chloride (NH4Cl), and 2.12g sodium bicarbonate (NaHCO3) per litre of distilled water [8, 11].

Leachate

The Municipal Solid Waste (MSW) leachate used in this study was obtained from a non-engineered active open landfill. It is located outside premises of Ahmadu Bello University, Zaria, Nigeria. The leachate was obtained by scooping from a low-lying open point at the three (3) different points in the landfill. The wastes generated are municipal solid waste from students, staff living off-campus and others which are dumped at the site.

B. METHODS

Test were carried out on the soil to determine the various bacteria present in the soil using microbiology test. Physico-mechanical and physico-chemical test such as specific gravity, the particle size distribution and Atterberg limits were used to characterize the physical properties of the soil sample used.

Microbiology Test

Pure culturess

In other to reducing microbial population, 1g of soil sample was dissolved in 9 ml of sterile distilled water to make soil suspension. Serial dilution was carried out for getting isolated single colony in which 1 ml of the dissolved suspension was drawn from the bottle and introduced to another sterile 9 ml of distilled water and mixed thoroughly and this was repeated for four different bottles; the 1 ml of the mix was draw out of the last bottle to remain 9 ml as the remaining bottle. These five bottles gave 1:10 dilution, 1:100 dilution, 1:1000 dilutions, 1: 10000 dilutions, and 1:100000 dilutions, respectively. In this research, nutrient medium was used for bacterial growth. 5.6 g of nutrient agar was dissolved in 200 ml distilled water, then the mixture was heated on a Bunsen burner and allowed to cool before it was poured into 10 petri dishes and oven- dried to solidify [13].

MICP Treatment

There are two methods of soil treatment namely, the injection method and premixing method. The injection method involves the flushing of bacteria solution on the sample from top down and a retention period is to observe for bacteria to be attached to the sand grains before injection of the cementation solution. In the pre-mixing method, the bacteria are mechanically mixed with the soil before the introduction of the cementation solution. Pre-mixing method was adopted in this study; where by natural soil was mixed S. pasteurii suspension density at optimum moisture content (OMC). The pore volume of the soil was determined then 1/3 of the pore volume was used to measure the volume of bacteria to be used on the soil for all treatment and suspension density at varying moulding water content. The equivalent 1/3 volume of the bacteria was deducted from the OMC then mixed together with water to amount to the total OMC then used to mix the soil sample together with water containing the 1/3 pore volume of S. pasteurii solution as recommended by (Soon et al., 2014) and allowed to stand for 12 hours to facilitate the

saturation of the S. pasteurii within the soil mass. Therefore, compaction was carried out in the moulds using British Standard Light (BSL) energies. Cementitious reagent was introduced into the soil until it was saturated. The cementation reagent was introduced on the surface of the soil and flowed through the soil by gravity. Repeated cycle of the cementitious reagent was done at six-hour interval for two days. Stepped densities of S. pasteurii suspensions densities were determined using MacFarland Turbidity scale using 0.5, 2, 4, 6 and 8 being equivalent to 0 cells/ml, 1.5 x 108 cells/ml, 6.0 x 108 cells/ml, 12 x 108 cells/ml, 18 x 108 cells/ml and 24 x 108 cells/ml respectively [14]. McFarland standard, which is equivalent to the bacteria suspension density, is shown in Table1.

Table 1: McFarland standard equivalent to bacteria suspension density Volume in mL.

Standard	1% BaCL2	1% H2SO4	Number of Bacteria cells/ ml/ (108) represented
0.5	0.5	99.5	1.5
1	1.0	99.0	3
2	2.0	98.0	6
3	3.0	97.0	9
4	4.0	96.0	12
5	5.0	95.0	15
6	6.0	94.0	18
7	7.0	93.0	21
8	8.0	92.0	24
9	9.0	91.0	27
10	10	90.0	30

Geotechnical tests

Natural moisture content

BS 1377 (1990) Part 2 was used to determine the natural moisture content of the soil obtained from the site. Three weighing containers were thoroughly cleaned and weighed to the nearest 0.01g (M1). The sample was crumbled and loosely placed in the containers, and the containers with the samples were weighed together to the nearest 0.01g as M2. The containers were then dried in an oven at 105-110oC for 24 hours. After that, the containers and samples were removed and weighed dry to the nearest 0.01g as M3. The natural moisture content (as collected on site) is calculated as the average of the three oven dried samples given by eqn. (1):

$$w = \frac{m_2 - m_1}{m_3 - m_1} X\ 100 \qquad (1)$$

Where w is the moisture content in percentage.

Specific gravity

Property	Quantity
Natural moisture content (%)	8.56
Percentage passing No 200	9.16
Liquid limit (%)	0
Plastic limit (%)	0
Plasticity index (%)	0
Linear shrinkage (%)	0
Specific gravity	2.63
ASHTO Classification	A-3(0)
USCS Classification	SP
Colour	Whitish brown

The specific gravity was determined using the BS 1377 (1990) [15] test (B) for fine–grained soils. The density bottle and stopper were weighed precisely to the nearest 0.001g (m1). The air-dried soil was placed in the density bottle, and the bottle, contents, and cover were all weighed as m2. After adding just enough water to cover the soil, the solution was gently stirred to remove any air bubbles. After that, the bottle was completely filled and covered. The covered bottle was then wiped dry, and the entire assembly was weighed to the nearest 0.001g (as m3). The bottle was then emptied and completely filled with water, wiped dry, and weighed to the nearest 0.001g (m4). The bottle was then emptied and completely filled with water, wiped dry, and weighed to the nearest 0.001g (m4). Equation 2 is used to calculate specific gravity:

$$G_s = \frac{m_2 - m_1}{(m_4 - m_1) - (m_3 - m_1)} \pi r^2 \qquad (2)$$

Particle size distribution

Particle size analysis was performed in accordance with BS 1377; 1990 Part 2. To obtain the particle size distribution, dry sieving was performed on 200g of soil sample. The same procedure was carried out on all S. pasteurii-treated soils.

Atterberg limits

The test consists of determining the liquid limits, plastic limits, and plasticity index for natural and stabilized soils. They were also carried out in accordance with BS 1377 (1990) Part 2 Test1(A) for natural.

IV. RESULTS AND DISCUSSION

Index properties of the natural and S. pasteurii treated soil are shown in Table 2 indicating the natural moisture content, particle size distribution, specific gravity.

Table 2: Index property of the natural and *S. pasteurii* treated soil

SIEVE ANALYSIS

PARTICLE SIZE DISTRIBUTION FOR THE NATURAL SOIL IS SHOWN IN FIGURE 1.

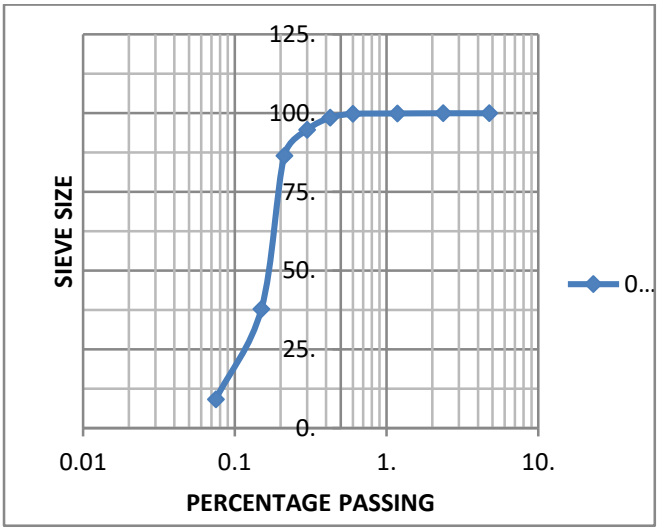

FIGURE 1: PARTICLE SIZE DISTRIBUTION CURVE FOR NATURAL SOIL

Fine are defined as the fraction of the soil particles that passes through the openings of the No. 200 sieve (0.075mm). Soils with very low fines typically have too little silt- and clay sized material to produce suitably low hydraulic conductivity. The dry sieving of the sandy soil treated with cementation reagent at various *S. pasteurii*suspension density ($1.5x10^8$cells/ml, $6x10^8$cells/ml, $12x10^8$cells/ml, $18x10^8$cells/ml and $24x10^8$cells/ml) for British standard light (BSL)[16] as shown in Figure 2.

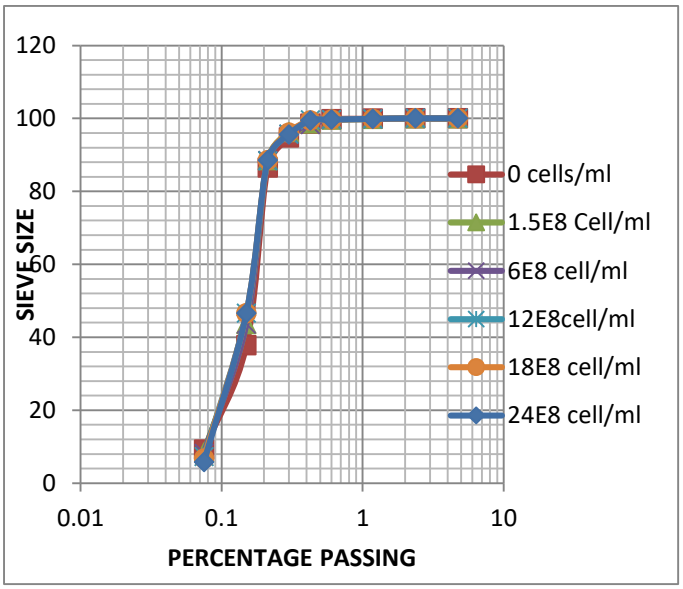

Figure 2: Particle size distribution curve for sandy soil – S. pasteurii suspension density treatment (BSL compaction energy)

Specific gravity

The variation of specific gravity of the natural soil and *S. pasteurii* treated with soil is shown in Figure 3. The specific gravity of the soil generally decreases with increased *S. pasteurii*suspension density. Its values decreased from 2.63 for the natural sandy soil to 2.35 at $24x10^8$cells/ml. this decrease may be caused by the proliferation of the *S. pasteurii* cells/ml as its suspension density increases. Also, the decrease can be attributed to the calcite formed during the MICP process which caused the soil particle to be flocculated within the soil matrix and hence particles are loosely parked [16].

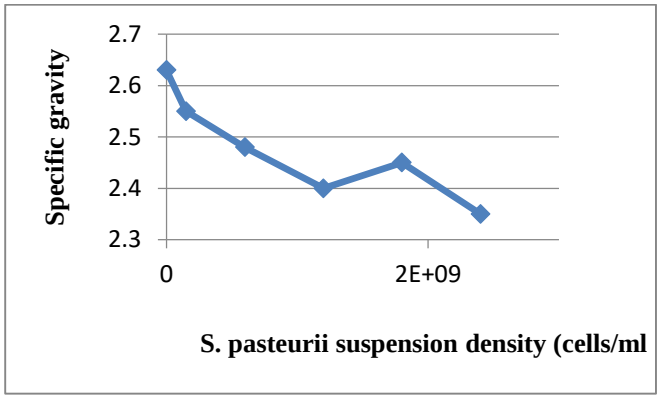

Figure 3: Specific Gravity

CONCLUSIONS

The natural soil has low moisture content of 8.56% because it was collected immediately after the rainy season. The atterberg limit of both natural and treated soil was nil which shows that the soil sample is a non-plastic soil. The specific gravity of the natural soil was found to be 2.63 which decrease to 2.33 at $24x10^8$cells/ml. The natural of soil particles passing BS. No. 200 sieve was found to be 9.16% decrease across the treated samples from 8.08% to 6.25%, 7.96% to 6.48%, and 8.58% to 6.02% at $1.5x10^8$cells/ml up to $24x10^8$

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