A SURVEY ON PRESENCE OF BIOPOLYMER PRODUCING MICRO ORGANISMS IN MARINE SOILS & ENHANCEMENT OF SOIL STRENGTH USING POTENTIAL ISOLATED MICRO-ORGANISMS: A REVIEW

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Abstract

High population densities are attracting towards coastal area due to which the demand of infrastructure is continuously increasing in that region. The properties of marine soil are very challenging for construction practice. There are some techniques to improve soil but due to their adverse effect on natural properties of soil, biological soil improvement method is introduced. The use of biological process in geotechnical engineering as a soil improvement technique has a potential to change soil properties. This is environmentally sustainable method. This paper represents the review on physical, chemical and biological properties of marine soil and enhance the strength of soil with the use of microorganisms. Plants are helpful in producing biopolymer naturally into the soil. Polyhydroxyalkanoates (PHAs) and cyanophycean can produce naturally by plants. There are different methods to use micro-organisms in soil such as MICP, biofilm, bio cementation, biopolymer. The isolation and screening of microbes were presented. There are many reactions occur in soil due to microbes and chemical composition in soil. some parameters such as concentration of microbes, curing time, type of biopolymer effect the unconfined compressive strength is also studied. Strength of soil is increases with increase in microbe's concentration and curing time. As the biopolymers are degrade with time by UV rays it generates Co_2 and H_2o. process such as swelling, hydrolysis, diffusion, erosion can control in plant field of plants. This biopolymer producing microbes use to reduce soil settlement, reduce liquefaction potential and increase water retention capacity.

Key words: marine soil microorganism, unconfined compressive strength, microbial reaction, biopolymer

I. Introduction

Marine soils are sensitive, having low shear strength and high compressibility. It has also problem of high settlement. (Aparna Sukumaran et. al. 2018). Marine soil has a relatively smoother surface and has finer particles. (Ben Li et. al.,2014). The strength and stiffness characteristics of marine soil deposits are subjected to wave induced cyclic stresses (Long & Menkiti 2007, Dias et. al., 2009; Milton S. da Costa et. al.2016, Hyde et al. 1993;).The physical properties of marine soil can know by performing different field and laboratory testing. Physical parameters include soil classification, specific gravity, MDD, Atterberg limits, relative density, unconfined compression strength, permeability. (Nipate Keshav V. et. al., 2015, Rakeshkumar dutta et. al.,2015, Aparna Sukumaran et. al.,2018). Heavy metals and other micronutrients are also present in soil. Atomic absorption spectrometry (AAS) is performed to identify them for chemical analysis. Chemical environment affects the biological process of soil and its treatment. (V. Sukumaran et. al.,2013, Munish kumar Mishra et. al.,2018)

There is different method to improve geotechnical properties of soil. The methods of treating soil with chemical and cement grout are widely used in geotechnical projects. But this method can damage the aquatic life, affect natural environment of soil and pollute groundwater flow. (Sourya Snigdha Mohapatra et. al.2020) To overcome this problem, environmentally sustainable method is required. So, from past 50 years attempt has been made to improve soil properties and strength by microorganism and biological process present in soil. (Pawar Shahaji P. et. al., 2015, Yusuf Abdulfatah et. al.,2018, Antonio soldo et.al.,2020) The soils have higher proportion of organic matters that acts as a cementing agent. (Basack S. et. all,2009). Formation of soil aggregates is a very complex process. (Katarzyna Szewczuk-Karpisz et. al.2019)

Some potential micro biological method uses to improve soil properties are mineral precipitation (MICP), mineral transformation, biofilms and biopolymers. (Edward Kavazanjian et. al.2008, Maria L. Auad et. al. 2020). The different microorganisms used for treatment produce chemically different binding agents (Aspiras R.B. et. al.,1971). Microbially induced calcium carbonate ($CaCO_3$) precipitation (biocalcification) is promising method of improving the geotechnical properties of soils (DeJong et al. 2010; Muynck et al. 2010; Chou et al. 2011). It results in increases in the soil's strength also. (DeJong et al. 2010; Whiffin et al. 2007; Al Qabany et. al., 2013). Biopolymer are organic polymer produce by microbes. Biopolymer treated soil has high durability and strength. This method is also economical. (Marta miletic et. al.,2020). To apply this method, first the identification of biopolymer producing microbes is very important.

To identify microbes, present in soil serial dilution method is use. All colony present in soil is cultured, isolate and screening by different method. (Nazia Jamil et. al.,2008 Charles E. Davidson et. al.,2014, Murtala Hassan Mohammed et. al.,2018). The temperature of site is measure at the time of sample collection. The microbes are cultivated at that temperature to get maximum growth. (Avishai Ben-David, Charles E. Davidson 2014). String test and Congo red agar plate test are used to check biopolymer producing potential of microbes. (Naveen Saxena et. al.2014). These biopolymer producing microbes produce polysaccharides. polysaccharides are sticky, thick biopolymer so it can easily bind particles and resulting in increased soil strength and reduce permeability (Jean Luc Chotte 2005). Some biopolymer such as Xanthan gum, Guar gum, Alginate, chitosan has potential to increase soil strength. (Antonio soldo et. al.,2020)

The biopolymer is able to some soil properties which help the growth of plant. Biopolymer change the permeability, structure, texture, transmissibility. Biopolymer can retain large number of water ant nutrients useful for plants in field. Some biopolymer can decompose toxic metal in soil and make soil suitable for plant's growth. (L. O. Ekebafe et al.,2011)

II. Materials and Method

This review paper is covered by five parts. First part is about the physical properties of marine soil which are determined in laboratory. Then the chemical analysis is done to find heavy metal and mineral present in soil. Also, it's concentration in soil is determined. The third part deals with biological study of soil. Biological study deal with isolation, screening and identification of microbes in soil by different laboratory method. Next part shows the relation and reaction between microbes and soil which can be used to change the soil's geotechnical properties. The last part gives details of different methods and techniques related to potential isolated microbes by which the strength of soil can increase.

2.1. Physical parameters of marine soil

Physical analysis of soil includes its engineering properties and index properties. Properties study by visualization are its colour, odour and texture. some index properties for clay are Particle size distribution, specific gravity, Atterberg limits (Mohammed Al-Bared,2017, Basack S. et. al 2009,). From particle size distribution curve, it was found that the soil consists of 14% sand, 27% silt and 59% clay, by weight. The value of Liquid limit is 89%, Plastic limit is 47% and Shrinkage limit is 16%. The plasticity index was as high as 42%. (S. Basack et. al,2009). The study conducted by Villarraga et. al. (2005) at Vishakhapatnam in India showed an increase in undrained strength around 20-100%, undrained stiffness around 50-100%, drained strength around 10-50 kPa and decrease in permeability of one to two orders of magnitude. Various tests are conducting on sand to determining various parameters like density, grain size distribution, MDD, coefficient of uniformity, coefficient of curvature, relative density. (Owais Hassan et. all,2020)

Strength of soil is measured my unconfines strength test, unconfined undrain triaxial test. Offshore structure is subjected to wave effect.so the cyclic characteristics is study by triaxial set up manually. The confining stresses in the triaxial compressive tests may destroy the bonding between weakly cemented soil particles. Unconfined compressive tests can determine the strength of soils without apply the confining stress. So, the bonding between particles is not affected. (Saxena and Lastrico, 1978). The unconfined compression test is suitable to find compression test of cohesive soil. The axial load was applied with a strain rate of 1.5%/min to determine compression strength. (ASTM) (Antonio Soldo et. al.,2020).

The series of Unconfined compression test on loose sandy soil grouted by zeolite and cement, the UCS appeared to be independent of the average size of the sand particles (D50). (Afshin Kordnaei et. all,2019, Pantazopoulos et all.,2012.)

For laboratory test, make uniform sand in three different grain size (D1, D2, D11). The sample of UCS was prepared at approximately 30 % relative density. The axial load was continuously increased at a strain rate of 0.3%/min. Unconfine compression strength of sample can determined with respect to D_{50} and active compound present in soil by given equation and as shown in figure 1 (Afshin Kordnaeij et. al, 2019).

$$UCS = 90.94 - 40.34D_{50} + 57.79AC^{1:665} \quad \text{(MRM equation)}$$

The undrained shear strength was measured in the laboratory by unconfined compression tests on specimens and by undrained triaxial tests on specimens from standard samples. Both disturb and remould strength is measured on three soil sample. (P. Lumb, and J. K. Holt, 1968). UCS test for sand is performed according to ASTM D2166/2166M-16(ASTM-2016) to measure the strength of given soil. (Maysam Bahmani et. al., 2019).The setup anf failure of specimen was shown in figure 2

The effect of salt present in soil-on-soil properties is studied by comparing the result of all test on natural and washed sample. Washed soil sample is prepared by mix moist soil with distilled water. Allow sufficient time for sedimentation and remove extra water without loss of fines. This process is repeated until sample is free from salt. Then the tests are performed on that washed sample. (Babu T. Jose et. al., 2008)

2.2. Chemical parameters of marine soil

Chemical analysis is important to study bacterial diversity of soil. The parameters study under these are: PH measure by PH meter, Electrical conductivity of soil by cation exchange capacity, nitrogen was estimated as described by (Subbiah and Asija (1956)),calcium was determined by Versenate method (Jackson, 1973), phosphorus by Brayl method as described by Bray and Kutz (1945),carbon content determined by Walkley and Black (1934), magnesium, sodium and iron were analysed by the method of Muthuvel and Udayasoorian (1999).Other micronutrients were study by Absorption spectrophotometer.(R. Kalaivani et. al., 2013) Heavy metals and chemical may also present in soil. It effects the growth of micro-organism and reaction in soil. It is important to identify them. This identification process completes by Atomic absorption spectrometry instrument (AAS). (Munir Kumar Mishra et. al., 2018)

AAS use the absorption of light by the elements in order to measure their concentration. The atoms present in sample absorb the Ultraviolet or visible light and transfer to higher energy level described by figure 3. By study the Absorption of these light the type of metal present and its concentration in sample is determine by detector. (moustafa Mohamed ahmed 2012). 1 gm of oven dry soil placed in 250 ml beakers to which 15 ml of aquaregia will be added. This mixture placed at 70^0 C until it become transparent. This solution filter with filter paper no.42 and dilute to 50 ml volumetric flask and diluted to mark volume using deionised water and the sample solution was use to find concentrations of Cu, Zn, Cd, Ni, Mn, Cr, Pb and other metals using an atomic absorption spectrophotometer (Perkin–Elmer).

The pH of the suspension was read using pH meter. Electrical conductivity is determined by Cation exchange capacity (CEC) Organic carbon content was determined by adopting chromic acid wet digestion method as described by Walkley and Black (1934), available nitrogen was estimated by alkaline permanganate method as described by Subbiah and Asija (1956) and available phosphorus by Brayl method as described by Bray and Kutz (1945). Available potassium was extracted from soil with neutral 1 N ammonium acetate (1:5) and the potassium content in the extract was determined by using flame photometer. (R. kalaivani and V. Sukumaran (2013)). As per V. Sukumaran et. al., (2013) paper, Other nutrient based parameters i.e., available phosphate and total nitrogen were estimated using standard methods of APHA (1987). Available micronutrients such as Zn, Cu and Mn were determined in the diethylene triamine Penta acetic Other nutrients such as magnesium, sodium and available iron were analyzed following the method of Muthuvel and Udayasoorian (1999).

The investigation was carried out on marine soil of thanjavur district of India ads the value of different parameters is as follow: pH (7.29-7.63), Electrical conductivity (0.26-0.52 Dsm-1), Organic carbon (0.42-0.60%), Clay (10.36-18.54), Nitrogen (84.2- 95.60Kg/ac), Phosphorous (3.25-3.75 Kg/ac), Zinc (0.74-0.89) Copper (4.85-8.23), Iron (0.78-1.05), Manganese (3.15-3.48)

X-Ray diffraction test is carried out to find mineralogical composition of soil sample. Then compare the observed X-ray pattern with standard JCPDS files, mineralogical analysis can carry out. The X-ray diffraction pattern of the soil indicated the presence of clay minerals, montmorillonites, chlorite, kaolinite, vermiculite and quartz. (S. Basack and R. D. Purkayastha, 2009). XRD analysis was carried out within a 10°–90° scanning range. SEM images were taken at 15 kV at different magnifications. (Meghna Sharma et. al., 2019)

2.3. Isolation, screening and identification of microbes

Microbes are naturally present into soil. Soil microbial populations play crucial role in soil properties. Microbial composition and functioning change the soil quality through decomposition of organic matter, recycling of nutrients (Christon stefanis et.al., 2013) but may be its not sufficient for improvement of soil properties. Hence, it requires isolate and screening of microbes to give them potential to change soil properties in eco-friendly way so the use of toxic chemical can reduce. (Sourya Snigdha Mohapatra et. al., 2007)

The simple method for isolation is serial dilution technique. Micro-organism culture on agar plate and developed different colonies. Number of colonies then transfer to single diluted plate for screening and identification (Avishai Ben-David et. al., 2014) For isolation, 5 gm of soil sample is suspended in 25 ml of Tryotic soy broth (TSB). These bacteria solution id diluted at 10^{-4} with saline water. These solutions transferred in three TSA plate to culture colonies. Gram staining technique is use to identify the microbes cultivated in agar plates. (Kohinur Begum et. al., 2017) One gram of soil from sample were suspended in 10 ml of distilled water and mixed for 15 minutes. Each suspension was serially diluted from 10^{-1} to 10^{-6}. Spread plate technique is used carried out to isolate the organism form the diluted sample. 0.1 ml was pipette out onto plates with nutrient agar and spread with a glass L shape rod and incubated at 37°C for 24 hours. The most prominent colonies were isolated and maintained at 4°C for further studies. Bacteria are found by its size, shape, arrangement and test of gram positive and gram negative. (Murugalatha N Kannan et. al., 2018)

There is different nutrient medium on agar plate. The reaction for B4 medium [yeast extract 4 g, glucose 5 g, and calcium acetate, Ca $(CH_3COO)_2$, 15 g/L of solution] are as below:

$$Ca\,(CH_3COO)_2 \rightarrow Ca_2 + 2CH_3COO^-$$

In B4a medium, calcium acetate was replaced by calcium chloride [yeast extract 4 g, glucose 5 g, and calcium chloride (CaCl2) 18 g/L of solution]

$$CaCl_2 \rightarrow Ca^{+2} + 2Cl^- \quad \text{(Paulo J. Venda Oliveira et. al., 2014)}$$

Quantitative evaluation of microbes is conduct by BT Technique. For this, selected isolated microbes is culture in four flask containing 50 ml of broth and urea of PH 8. The flask is heated at 28^0 C and shaken at 150 rpm speed for calcite production and bacterial growth. The growth of bacteria is observed by using Nikon SMZ10 Stereomicroscope after 48 hours. Then confirm the growth of samples. Then pour the flask material into 50 ml falcon tubes and centrifuge it three times for 10 minutes with acceleration of 6024g.The residue of that contain calcium carbonate precipitation. The back-titration technique is used to measure the quantity of calcite precipitation after drying the biomass. (Maysam Bahmani et. al., 2019)

The isolate colonies were identified or classify on basis of temperature, PH, different sugar utilization, hydrolysis test, protein utilization and morphologic study. (Anupama sapkota et. al., 2020) EPS is biopolymer which has properties like thickening, gelling etc. (Upma Dev et. al., 2018) EPS has capacity to aggregate the soil particles. it can act like a glue, getting attached to clay and ions, holding solid particles together (Chenu,1995). Congo red agar test shown in figure 4 and tube test is used to check the presence of EPS and its capacity. (Naveen Saxena et. al., 2014)

2.4. Interaction between microbe and soil

There are approximately 10^9 to 10^{12} organism present in soil near ground surface. Organism may include bacteria, virus, fungi, archaea, and eukarya, protozoa, algae and slime molds. microbes absorb energy during oxidation and chemical compound gave electron. The oxidation-reduction process happens simultaneously in soil. Due to this biochemical process randomly oriented clay-silt particles bond together tightly. (J. Carlos Santamarina et. al., 2005)

Fungi can decompose the organic matters into soil and the structure of soil is damaged and it's also effect soil aggregation. Fungi can produce hydrophobic compound in soil which

+can affect infiltration properties of soil. (Karl ritz et. Al., 2004) introducing a fungus did not cause a reduction in the quantity of macroaggregates with respect to the control soil. This shows the role of fungi in the stabilization of aggregates (Tisdall et. al. 1982; Miller et. al., 1990; Beare et al. 1997; Tisdall et al. 1997). Most of micro-organism produce polysaccharide. They have electrostatic charge on its surface so it attracts the soil particles and bind them together. Due to this binding soil aggregation can occur and soil structure can change. (P. A. Maclean et. al.,1978)

Some micro-organism changes structural Fe (3) to Fe (2), by this smectite transform to illite. Smectite promote to excessive swelling where Illite have much lower swelling potential. This transformation may take up to 14 days. (Edward Kavazanjian et. al.,2008)

The scanning electron microscope (SEM) is use to see the bond between soil and biopolymer. The coating agent surrounding the soil particles can visible. The range of magnifying can change to see more cleared image. By SEM image it observed that the biopolymer act as coagent agent for soil which was visible in figure 5.The biopolymer and soil connect to each other by electrostatics bond. (Antonio Soldo et, al.,2020)

The diverse microorganism's presence in soil can produce chemically different binding agents (Aspiras et al., 1971). Different binding agent can affect different properties of soil. (R. F. Harris et. al.) The effect of temperature on stability metabolic activity (Harris et al., 1966a; Martin and Craggs, 1946).1 mole of urea decomposes into 2 moles of ammonium according to following reaction:

$$CO\,(NH_2)_2 + 2H_2O \rightarrow 2NH^{4+} + CO_3^{2}$$

NH^{+4} increase PH which is suitable environment for microbial calcium precipitation. Ca^{+2} present in soil make product with remain co_3^{-2} in above equation. The $CaCO_3$ formed are gelation in nature and it can bind the particles of soil.

$$Ca^{2+} + CO_3^{2-} \rightarrow CaCO_3$$

Organic nitrogen in form of NH_3 release in soil by organic compound. It reacts with soil and produce alkalinity which leads to increase in PH.

$$H_2O + NH_3 \rightarrow NH_4 + + OH^-$$

In this condition NO_3^- can use as electron acceptor by many microorganisms. Sulphate can also use as electron acceptor.

$$NO_3^- + 1.25CH_2O = 0.5N_2 + 1.25CO_2 + 0.75H_2O + OH^-$$
$$SO_4^{-2} + 2CH_2O = H_2S + 2CO_2 + 2OH^-$$

(Krumbein, 1979; Stocks-Fischer et al., 1999; Fujita et al., 2000; Hammes et al., 2003; Whiffin, 2004).

2.5. Enhance soil strength by microbes

Many bacteria produce a layer of polysaccharides or polyproteins with help of plants which coats the soil particle's surface.It's played an important role in cementing the sand, clay, silt particles and convert in microaggregates that improve the soil strength. (James. J. Hoorman, 2016)

Utilize the knowledge of civil engineering, chemistry and biology to improve the strength of soil. (Whiffin et al., 2007; Ivanov and Chu, 2008; Mitchell et. al., 2005;).Micro-organism can change the behaviour of course graine soil and influence the formation of fine-grained soil. It creates chemical reaction with soil and change the chemical and mechanical behaviour of soil including soil strength. (Santamarina et. al., 2005). Microbial induce chemical precipitation can change the characteristics of soil such as strength, stiffness and permeability. It also changes hydraulic conductivity.

Murtala Hassan Mohammed et. al. (2018) prepared bacterial solution at 3 different concentration 1×10^5 cfu/ml, 1×10^6 c fu/ml and 1×10^7 cfu/ml by series dilution technique. cementation reagent such as urea, calcium chloride add into a litre of water and solution of MICP is prepared. It mixed with soil sample at 25 % water content. It can increase unconfines strength up to 17%. Bacterial concentration effects the increase in strength of soil.

The Bacilluspasteurii bacteria was cultured and special agar is prepared by it to change the soil properties. After treated the soil with bacterial solution, strength of soil is measured by box shear test at periodically. The graph represents that with increase in time period and normal stress, coefficient of shear stress is also increased. (Pawar Shahaji et. al.,2015). The effect of bacteria on soil properties change is depend on co_2 produce by bacteria. For these Bacilluspasteurii type bacteria is most suitable to change Atterberg limits and unconfined strength of soil. Atterberg limit and UCS was performed on soil sample before treatment based on IS-2720 part 5,6,10. After treated with bacteria, average decrease in liquid limit is 36%, average decrease in plastic limit was 12.4 % and increase in UCS was 80 %. (Sheela Evangeline Yasodian et. al.,2012).

The soil sample are tested for UCS to determine the compressive strength after treatment. Antonio Soldo et. al. (2019). Determine the UCS of one soil by adding five different type of biopolymer: Xanthan Gum (XG), Beta 1,3/1,6 Glucan (BG), Guar Gum (GG), Chitosan (CHI), and Alginate (ALG). Figure 6 shows the influence of different types and different concentrations of biopolymers on the compressive strength of the wet specimens which were tested within an hour of the preparation. (Antonio Soldo et.al.,2020)

Biopolymer are biodegradable product due to this it uses as temporary support system in soil excavation. Biopolymer make gel into soil which increase liquefaction resistance and strengthen the soil. The CL-ML type soil is treated with three different type of biopolymer such as Xanthan gum, sodium alginate, and slime forming bacteria with 2% dosage. The strength after the treatment is measured by triaxial test. The shear strength was increased about 50% during the first week. (James K. Mitchell et. al., 2005). Very small amount of Xanthan Gum in sand affect the strength behavior of sand. Increase the content of Xanthan gum can increase the cohesion but the friction angle remains constant. Gellan Gum Can increase the soil strength and soil durability by thermogeletion treatment. Guar gum can increase the soil strength. Agar can also affect the shear strength of soil due to curing time effect as shown in figure 7. This all biopolymer tends to increase cohesion in soil but the friction angle of treated soil is decrease with time. (Jung Yeon Jung, 2020). Alginate and cellulose are also one type of biopolymer which is used in soil to change its properties. The exopolysaccharide and capsular-polysaccharide are different type of biopolymer made from microbes. They are soluble in water and this solution is used in soil to increase its strength. (Bernd H. A. Rehm,2010).

III. Results and Discussion

The samples were prepared in same manner and test were performed on it. The range of their values are presented in this chapter.

3.1 Results of physical test on marine soil (Before treatment)

Table 1. shows the value physical parameters of soil such as liquid limit, plastic limit,specific gravity,OMC, MDD, UCS without treatment and range of their values

3.2 Results of chemical test on marine soil (Before treatment)

Table 2 represents the chemical parameters of soil such as present of heavy metals, hazardous compound etc.

3.3 Result of increase in strength of marine soil by microbes

The study shows that soil microorganisms play an important role in the aggregate formation and stabilization processes of soil. polysaccharides enable themselves to be adsorbed on clay particles and also to glue particles together. Change in soil strength depend upon the type of polysaccharides used for treatment. The bacteria type microorganism Bacillus pasteurii is effective for increase strength of soil. Biopolymers, such as Guar Gum (GG), Xanthan Gum (XG), Chitosan (CHI), and Beta 1,3/1,6 Glucan (BG) can improve engineering properties of marine soil. The properties are change due to aggregate of soil and change in soil structure after treated with biopolymer producing microbes.

Clay content is reduced about 30-47 % and silt content is increases about 29-48 %. Due to aggregation of soil. Plasticity index can reduce approximately 24 %. The unconfined compressive strength can increase about 80-190%.

Figure 8 show that as the bacterial concentration is increase, calcite content is also increasing and with that the strength of soil increases. The strength and calcite content are also increasing with respect to time.

The environmental conditions during the curing and testing phases effect the strength of marine soil. Daily change in temperature and humidity is not suitable for growth of microbes. The different microbes can survive in different environmental condition so if they don't get suitable condition, they can't show maximum growth. It directly affects the change in soil strength.

IV. Conclusion

Introducing biopolymer producing microbes is considered as successful technique to improve strength of marine soil. Increase in strength depends on Temperature, concentration of microbes, available salt content, type of plants on field. The presence of chemical component in soil also effect its strength. If environment condition is suitable to microbes, it will show maximum growth, so the increase in strength is higher. It also effects the stiffness and settlement of marine soil. Strength increases continuously with time after adding microbes. Biopolymer can increase water retention capacity of soil, so demand of water for plant growth will reduce.

Figures and tables

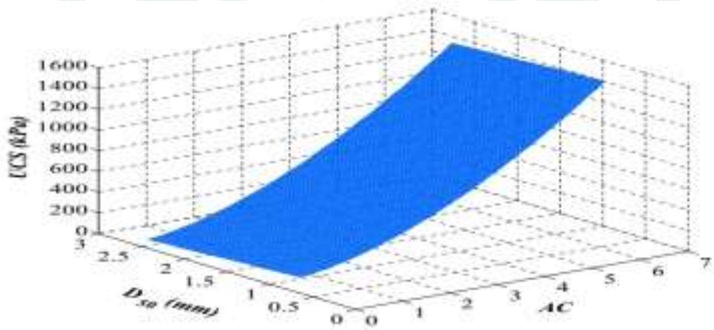


Figure 1. Correlation of UCS with AC and D_{50} using proposed MRM equation (Adopted from Afshin Kordnaeij et. al, 2019)

(a) (b)

Figure 2. (a) An experimental setup for UCS test. (b) sample of UCS after test (adopted from Banita behera, M. K. Mishra, 2012)

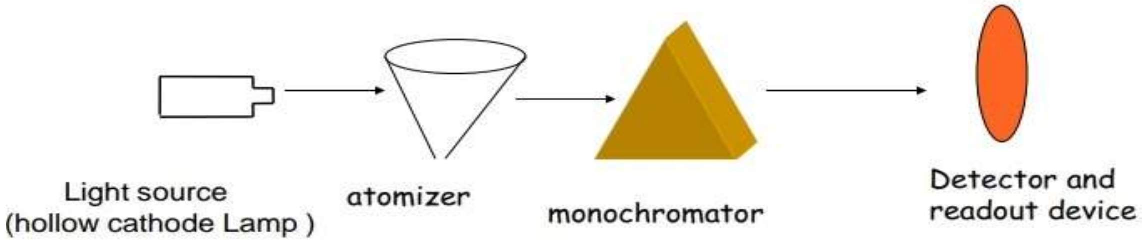
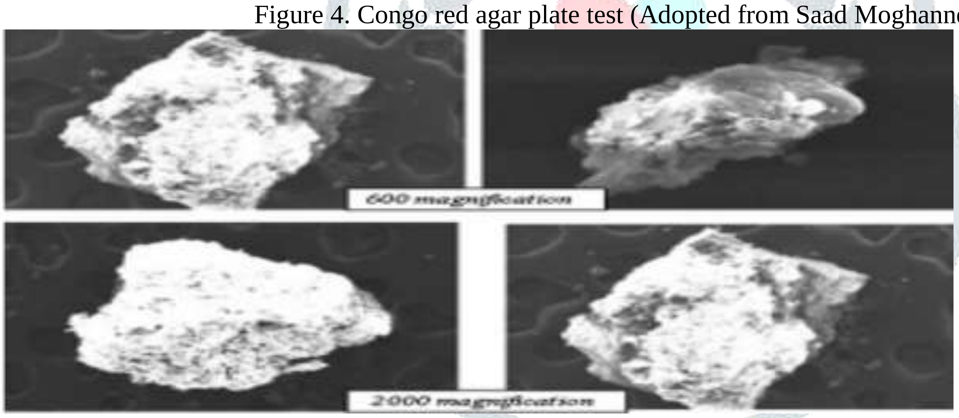


Figure 3. Schematic diagram of AAS (adopted from Moustafa Mohamed Ahmed 2012)

Figure 4. Congo red agar plate test (Adopted from Saad Moghannem)



(a)

(b)

Figure 5. SEM Image of soil sample (a) untreated soil sample (b) treated soil samples (Adopted from Rakeshkumar Dutta 2012)

Figure 6. Change in compressive strength under the influence of time, biopolymer-type, and biopolymer concentrations. (Adopted from Antonio Soldo et.al.,2020)

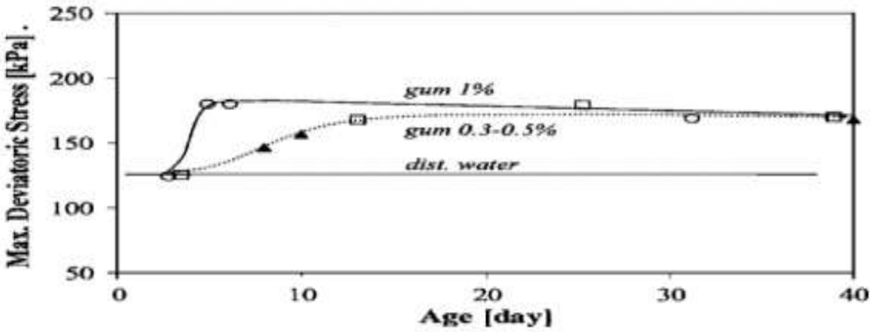


Figure 7. Effect of xanthan gum and Na- alinate treatment on strength of CL-ML soil (adopted from martin et. Al.1996)

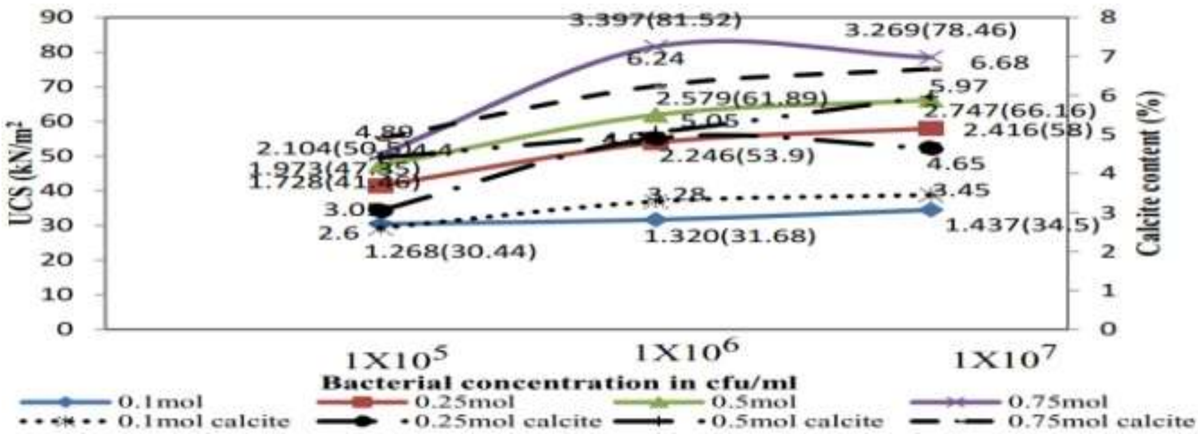


Figure 8. Increase in UCS value and calcite content with bacterial concentration after 48 hours

Table 1. Physical parameters of soil and range of their values

Parameters	Range of values
Liquid limit (%)	76-120
Plastic limit (%)	44-85
Shrinkage limit (%)	14-31
Plasticity index	32-55
Specific gravity	2.4-2.62
Optimum moisture content %	17-25
Maximum dry density gm/cm^3	1.60-1.73
Unconfined compressive strength (KN/m^2)	4-5.6

Table 2. Presence of chemical constituent in soil and range of their values

Parameters	Range of values	Parameters	Range of values
PH	7.29-7.63	Potassium(kg/ac)	125-145
Electrical conductivity (dsm^{-1})	0.26-0.52	Zinc(ppm)	0.74-0.89
Organic carbon %	0.42-0.62	Copper(ppm)	0.78-1.05
Nitrogen(kg/ac)	84.2-95.6	Iron(ppm)	4.85-8.23
Phosphorus(kg/ac)	3.25-3.75	Heavy metals	Presents

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