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Influence of Crude Oil Contamination and Bioremediation on Geotechnical Properties of Marine Sand

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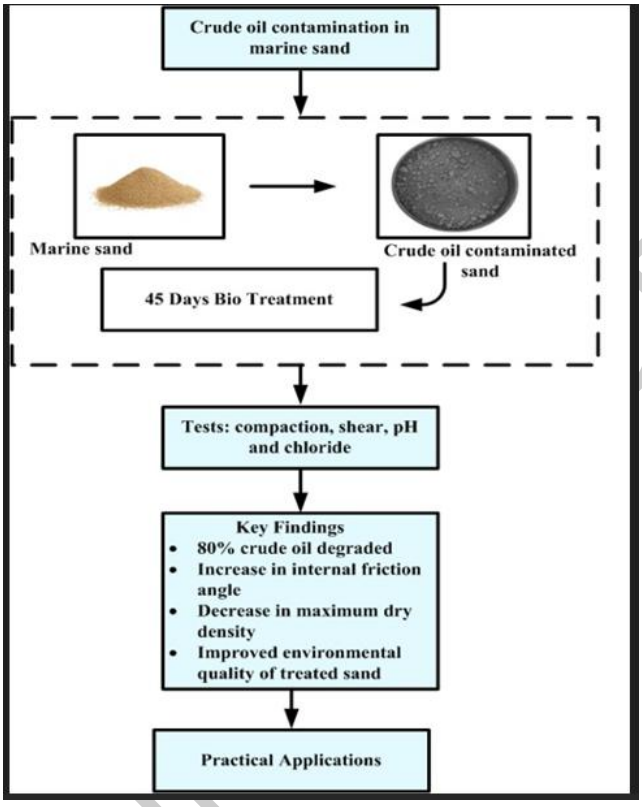
Graphical abstract



Abstract

The contamination of marine sand with crude oil (CO) can significantly alter its geotechnical properties, including its strength, compressibility, and permeability. The bioremediation process, which uses microorganisms to break down and eliminate toxins, can alter the structure and composition of the soil, which can further affect these characteristics. Various oil-degrading bacteria have been proven to remove oil contaminants from soil. Their impact on the geotechnical characteristics of polluted materials is not well studied, nevertheless. A bacterial strain called Bacillus amyloliquefaciens, with concentrations ranging from 2% to 12% is used in this study to break down oil pollution from crude oil-contaminated sand. The change in the bioremediation sand's geotechnical characteristics was then determined. Results showed that after 45 days of treatment, up to 80% degradation of crude oil was achieved, with higher bacterial concentrations correlating with increased degradation efficiency. The angle of internal friction increases with treatment duration and bacterial concentration, while the highest dry density decreases with crude oil concentration and chloride content. These findings demonstrate that controlled bacterial treatment not only mitigates contamination but also enhances soil properties, supporting the potential use of biotreated marine sand in offshore foundation construction and as a stable road base material, subject to further field validation.

Keywords: Geotechnical properties, Crude oil contamination, Soil stabilization, Bio treatment, Marine sand.

1. Introduction

The global consumption of crude oil was 102.21 million barrels per day in 2023. Estimates indicate that by 2024, economic activity and the associated demand for oil might increase to about 104 million barrels per day. By 2045, OPEC predicts that the demand for oil products worldwide will amount to 110 million barrels per day.(Vickery and Cutler), There are risks associated with moving oil from production facilities to areas for consumption, most notably the possibility of unintentional oil spills that might harm ecosystems and endanger human society. Globally, there is a forecasted significant growth in inter-regional trade in oil in the coming decades. Learning from the past is essential to preparing for oil leak disasters. However, this task is challenging because the consequences of such disasters are contingent upon the specific spatial and temporal contexts in which they occur. It is imperative to adopt efficient methodologies to articulate accurate assessments regarding the potential impacts, including environmental damage, economic losses, impacts on human health, and harm to wildlife, of future oil spill mischances (Chang *et al.* 2024). Oil spills occur due to a

variety of reasons, which can be broadly categorized under two main heads: human mistakes or system glitches (Devatha *et al.* 2019). This causes major issues since it contaminates soil, breaks down the soil's structure, interferes with biodegradability, and endangers both human and ecological health (Kaplan *et al.* 2022). Crude oil pollution poses concerns to the environment, but it may also drastically change the geotechnical characteristics of the contaminated soil, causing serious harm to already-existing buildings. This has led several academics to concentrate heavily on examining how pollution from crude oil affects the geotechnical characteristics of soils.

The combination of hydrocarbons that make up crude oil is very viscous and thick, including both bigger, non-volatile components and smaller, volatile ones. The primary elements in these hydrocarbons are hydrogen and carbon, followed by nitrogen, sulphur, and oxygen. Additionally, crude oil contains traces of nickel, chromium, and vanadium (Muthukumar *et al.* 2021).

Bioremediation, the microbial degradation of hydrocarbons, is a convenient, sustainable, cost-effective, and environmentally friendly method. Numerous bacteria possess the capability of digesting hydrocarbons for their energy, making this process an effective solution for cleaning up contaminated environments (Katukojwala *et al.* 2021).Several works were suggested in the literature related to the effect of Oil-Contamination on the geotechnical properties of soil and works related to bioremediation of such contaminated soils. A few recent works are as follows. Bioremediation of oil-contaminated soil was the process of adding nutrients to the soil to increase the microbial population (biostimulation) and decompose the pollutants (bioaugmentation). Developed in the 1940s, the bioremediation technique only became well-known in the 1980s as a outcome of the infamous Exxon Valdez oil disaster (Soltani-Jigheh *et al.* 2018; Fingas and Fieldhouse2012). A field investigation was carried out in 1994 to ascertain whether the biosurfactant PES-51 was successful in extracting weathered CO from contaminated sand from the Exxon Valdez oil spill at La Touche Island, Prince William Sound. All of the diesel range oil was removed below the 0.5 mg/kg detection level, per the results of the investigation. 70% of the semi-volatile components were also removed by the biodegradation (31). Individual bacterial cultures and the planned bacterial consortium's effectiveness in degrading crude oil were evaluated by Rahman *et al.*, (2002). Out of 130 oil-degrading bacterial cultures, five strains (Corynebacterium sp. GS5-66, Pseudomonas sp. DS10-129, Micrococcus sp. GS2-22, Flavobacterium sp. DS5-73, Bacillus sp. DS6-86) were chosen for the investigation because of their effectiveness in breaking down CO.According to the results, after 20 days of sampling, a mixed bacterial consortium consisting of these bacterial strains had an oil breakdown efficiency of up to 78%.Furthermore, Singh *et al.* (2012) found that the application of microbial consortia reduced the petroleum pollutant in soil from 30.9% to 0.97% after 360 days of treatment, whereas the control plot only had a 5% drop. Bacillus, pseudomonas, Acinetobacter, and Azamines were the most often utilized degrading bacteria to eliminate petroleum pollution from soil etc (Sanders 2012; Khamehchiyan *et al.* 2007). Bacillus subtilis and pseudomonas fluorescence with a composite used as oil degrading agents to degrade engine oil concentration and improve strength parameters in clay soil. Acinetobacter calcoaceticus with other bacterial strains in natural soil, was identified and used to decrease crude oil concentration and also improve geotechnical properties in clay soil. Bacillus endospores with organic and chemical nutrients help to minimize the effect of engine oil contamination in marine sand and alter the compaction and shear strength. Bacterial organisms isolated from contaminated sites were used as remediation materials, which help to decrease the crude oil concertation and improve strength properties. (Puri *et al.* 1994). According to the literature cited above, there have been several studies on how CO contamination affects the geotechnical characteristics of contaminated soil as well as some on how to ameliorate the geotechnical characteristics of soil polluted by crude oil. Among other bacterial groups, bacillus has a high oil-degrading capacity (Shin *et al.* 2002). Bacillus amyloliquefaciens was used separately and along with other bacteria in various studies, and gives a good reduction oil concentration. However, no substantial research has examined how these oil-degrading bacteria affect the geotechnical characteristics of soil polluted by crude oil.

1.1. Novelty

The novelty of this work lies in the targeted application of Bacillus amyloliquefaciens for the bioremediation of CO-contaminated marine sand, with a simultaneous evaluation of changes in its geotechnical properties. While previous studies have illustrated the oil-degrading ability of Bacillus amyloliquefaciens either individually or in combination with other bacteria, these works have largely focused on soil or terrestrial environments without addressing marine sand conditions. No substantial research has systematically investigated how this specific bacterial strain alters the strength, compressibility, and permeability of crude oil-polluted marine sand during and after biotreatment. This study is unique in bridging the gap between microbial bioremediation efficiency and post-treatment geotechnical suitability, highlighting the potential for reuse of biotreated marine sand in offshore structure construction and road base applications.

The primary aim of this research is to examine how CO pollution affects the geotechnical characteristics of sea sand and how well the bacterial strain Bacillus amyloliquefaciens performs bioremediation.

2. Materials and methods

2.1. Marine sand

Soil was gathered from Ganagalla Peta beach, Andhra Pradesh at a latitude and longitude of 18.21° N and 83.95° E. The sample was taken at a depth of 0.3m and transported to the laboratory. A total of **50 independent**

soil samples were prepared for the experiment: **25 oil-contaminated samples** and **25 bioremediated samples.** There were no visible signs of oil contamination at the location where the soil sample was taken.

Table 1. Properties of soil sample

SL.NO	PROPERTY	VALUE	IS METHOD
1	Specific Gravity	2.65	IS 2720PART31980
2	Medium Sand	0	IS 2720PART41985
	Fine Sand	63.57%	
	Silt	35.91%	
	Clay	0.17%	
	Uniform Coefficient, Cu	1.84	
	Coefficient Of Curvature Cc	0.98	
3	Is Classification	SP	IS 2720PART41970
4	Angle of internal frictionat a density of 1.65g/cc	33°	IS2720PART131986

The mechanical sieve analysis, which complied with IS 2720-part 4, determined the proportion of different sized particles in the sand. **Table 1** displays the results of the sieve study, and **Figure 1** displays the distribution curve for particle size.

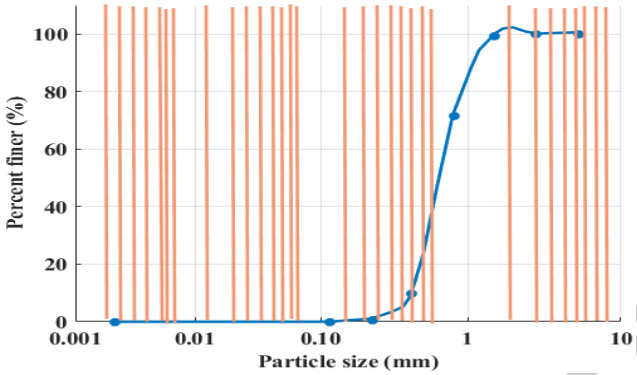


Figure 1. Particle size distribution curve of Marine sand

2.2. Crude oil

Unprocessed oil, known as crude oil, was gathered from Chennai Petroleum Corporation Limited at Manali, Chennai. Crude oil is chosen because it has a high possibility of contamination at the seashore. The oil underwent testing at a laboratory temperature of 28 ± 1•0°C. **Table 2** displays the Properties of crude oil.

Table 2. Properties of crude oil

Parameter	Quantity
Viscosity (gm-1 s-1)	45
Density (g/cm3 at 15C)	0.923
API gravity at 60F)	21.4
Flash point(C)	48
Specific gravity (at 25C)	0.8585

2.3. Bacteria

Gram-positive, rod-shaped, endospore-forming Bacillus amyloliquefaciens is a member of the Bacillaceae family. It is non-pathogenic, human friendly, and has potential use in the agricultural field.

3. Methods

3.1. Bacterial solution

Bacteria in a viable condition have grown in culture media, which is necessary for nutrition. After attaining the required growth, the bacterial culture is used to degrade the oil concentration in marine sand. A Bacterial solution of Bacillus amyloliquefaciens is prepared by following the procedure.

3.2. Bacterial cultivation

Bacterial culture is one method that makes it possible for bacterial cells to develop in or on a culture medium under closely watched laboratory settings. The required concentration of bacterial solution has been prepared by the cultivation process.

3.2.1. Streak Plate Method

Soil sample preparation and enrichment, Bacillus amyloliquefaciens was cultivated and enumerated using standard microbiological methods to ensure isolation of pure cultures and accurate determination of viable counts. For pure culture isolation, the streak plate method was employed. A sterile inoculating loop was used to transfer a small amount of bacterial suspension onto the edge of a sterile nutrient agar plate. The inoculum was streaked across the agar in three sequential sectors, sterilizing the loop between each sector to progressively dilute the bacterial concentration. This method ensures that only a few bacterial cells remain on the loop in the final sector, allowing single cells to develop into discrete colonies upon incubation at 35–37°C for 24 hours (Ratnaweera and Meegoda 2006). **Figure 2** shows the development of Bacillus amyloliquefaciens colonies by the streak plate method.

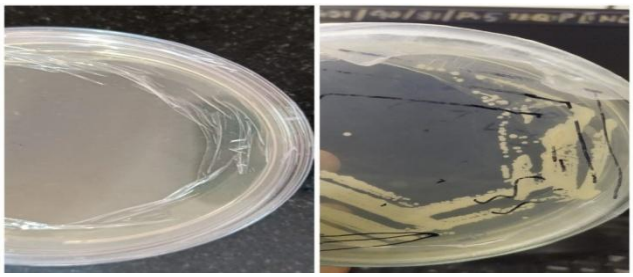


Figure 2. Cultivation of bacillus amyloliquefaciens – streak plate method

3.3. Colony forming unit

In this process have three steps

They are, 1. Serialdilution 2. Spreadplating 3. Colonycounting

3.3.1. Serial Dilution

Serial dilution is a microbiological technique used to progressively reduce the concentration of bacterial cells in a sample, ensuring an appropriate cell density for accurate colony enumeration. As shown in **Figure 3**, 7 sterile test tubes were prepared, every containing 900 µl of sterile diluent (distilled water). Utilizing a sterile micropipette 100 µl of a well-mixed bacterial culture from the previous step was transferred into the first tube, bringing the total volume to 1 ml and producing a dilution factor of 10^{-1}. The suspension was mixed thoroughly by pipetting several times to ensure homogeneity. After that, a sterile pipette tip was employed, and 100 µl of the 10^{-1} dilution was moved into the second tube, which held 900 µl of diluent, creating a 10^{-2} dilution. This step was repeated sequentially for all seven tubes, generating a dilution series from 10^{-1} to 10^{-7}, equivalent to a concentration reduction of 1 in 10,000,000 (Khosravi *et al.* 2013). The prepared dilutions were then used for subsequent spread plating and colony counting to determine viable bacterial counts.

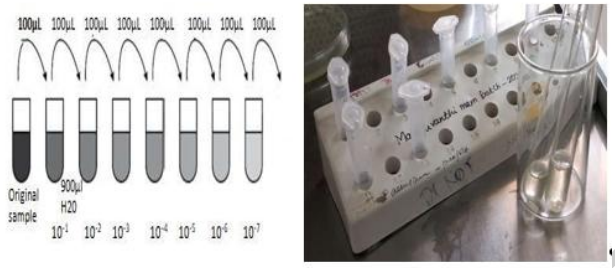


Figure 3. Model diagram of serial dilution method

Figure 4. Spreading on agar surface

3.3.2. Spread plating

The spread plate method was used to evenly distribute diluted bacterial suspensions onto the surface of solid nutrient agar for colony development, as illustrated in **Figure 4**. Following the serial dilution process, aliquots from the last three dilutions were selected to ensure countable colony ranges. Using a sterile micropipette 100 µl of each selected dilution was dispensed onto the center of a sterile nutrient agar plate. A clean glass spreader was then dipped into a beaker containing ethanol, briefly flamed to sterilize, and allowed to cool to avoid heat damage to the inoculum. The cooled spreader was used to gently and uniformly spread the inoculum across the entire agar surface in a circular motion to ensure even colony distribution. The plates were then incubated at 35–37°C for 24 hours to allow visible colony formation (Ghaly 2001).

3.4. Colony Counting

Following incubation, bacterial colonies were enumerated using a digital colony counter, as shown in **Figure 5**, to determine the count of colony-forming units (CFU) in the original sample. Each plate was placed on the illuminated stage of the colony counter, where transmitted light enhanced colony visibility. Colonies were manually marked using a specialized pen integrated with the device, which electronically recorded each count and displayed the total on a digital screen. This method ensures accurate enumeration by magnifying the plate surface and reducing counting errors. Assuming that every visible colony was the result of a single viable bacterial cell, the CFU per millilitre of the original culture was computed by multiplying the number of colonies by the inverse of the dilution factor (Jukic 2013).

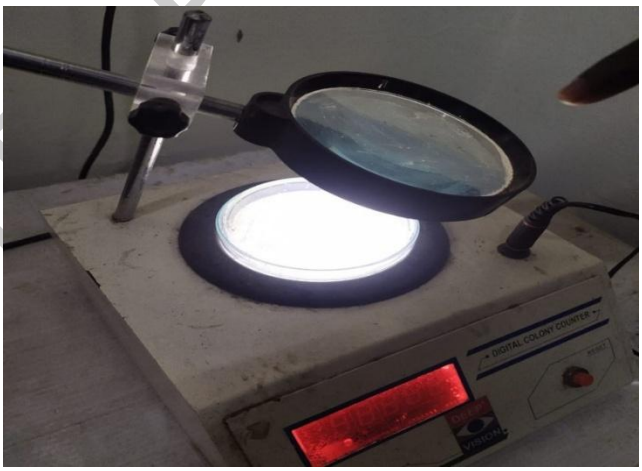


Figure 5. Colony counting apparatus.

The CFU/ml can be calculated using the formula:

$$CFU\ /\ ml = \frac{(no.\ of\ colonies \times dilution\ factor)}{Volume\ of\ culture\ plate}$$

3.5. Preparation of media

Culture media, often referred to as growth media, are certain combinations of nutrients and other materials that promote the development of microorganisms such as moulds, yeasts, fungi, and bacteria. The creation of a microbiological medium does not eliminate the need for sterilization due to microbial contamination from hands, glassware, air, etc. We use the autoclave, which is essentially a massive steam cooker, to sterilize media.

Culture media, which can be semi-solid or solid, are often made in petri dishes using a nutrient broth (liquid) that has been combined with agar. The autoclave sterilisation settings are 121°C for 15 minutes at >15 psi. For most species, the thermal death period is fifteen minutes

(Fallah *et al.* 2015). Sterile agar media prepared for bacillus amyloliquefaciens.

3.6. Preparation of Bacterial Culture

For each bacterial strain, Spread plating was done, and the amount of bacterial colonies per ml of culture was determined by the colony counting method. The bacterial culture was scaled up to the necessary amount using a shaker at 35^{o}C to 37^{o}C. The formation of bacteria takes place in the culture media, as 4% 6% 8%, and 10% variations applied to the sand sample. The quantity of bacterial culture for each percentage was calculated as each milliliter contains a concentration of 10^{7} CFU.

3.7. Soil sample preparation

Soil sample preparation contains two different stages 1. oil-contaminated sand preparation and 2. bio-remediated sand preparation. The first step involved sterilizing the soil by autoclaving it at 115 degrees Celsius for 15 minutes. They then sprayed 2%, 4%, 6%, 8%, 10%, and 12% of CO by dry weight of soil specimen into the soil sample and blended by hand to generate a homogenous mixture. The mixture was put in an airtight plastic container and kept in laboratory conditions (temperature 28ºC, 1.123 atmospheric pressure) up to the test date. During the preparation of bio-remediated sand, 2%,4%,6%,8%,1and 0% of the bacterial solution by dry weight of soil mixed and sprayed on oil contaminated sand. 10 g per kg of powdered cow dung is added periodically to the mixture. A temperature range of 26°C to 29°C and a pressure of 1.019 atmospheres were maintained throughout the treatment. Samples were mixed every two days to provide aeration and control the values of salinity, pH, and relative humidity.

Properties are varied for different oil percentages, different percentages of bacterial solution, and different time durations. Tests are carried out for 7, 14, 30, and 45 days to ascertain the characteristics of uncontaminated, oil-contaminated, and bioremediation sand compaction (IS2720:1974 PART 8), direct shear (IS2720:1974 PART 13), electrical conductivity (IS2720:1987 PART 26), and pH (IS14767:2000). FTIR analysis is done to measure hydrocarbon reduction which performed using IRSPRITxseries model.

4. Results and discussions

4.1. Compaction test

The significance of compaction qualities in the road building sector makes them extremely significant. Compaction test apparatus for light compaction AIMIL (AIM 110).

To ascertain the impact of crude oil on the compaction behaviour, both clean and contaminated soil samples were subjected to standard Proctor compaction tests by IS 2131 (1981). One may use Is 2720-2 to determine the moisture content in clean soil:

$$w\% = \left(\frac{Ww}{Ws}\right) \times 100\% \quad (1)$$

where Ww specifies the weight of the water, ωspecifies the moisture content, and Ws specifies the soil solids weight. Because the moisture content cannot be determined using Equation (1) when there is oil contamination present, the procedure of Khamehchiyan *et al.*, as displayed in Eq. (2), was applied to estimate the moisture content for all oil-contaminated samples:

$$w\% = (1+mn)\frac{Wt}{Wd} - (1+n) \quad (2)$$

here, Wd and Wt are the dry andwet weights of contaminated soil, correspondingly; m (%) is denoted as the CO residual content after drying and n (%) is the crude oil content before drying.

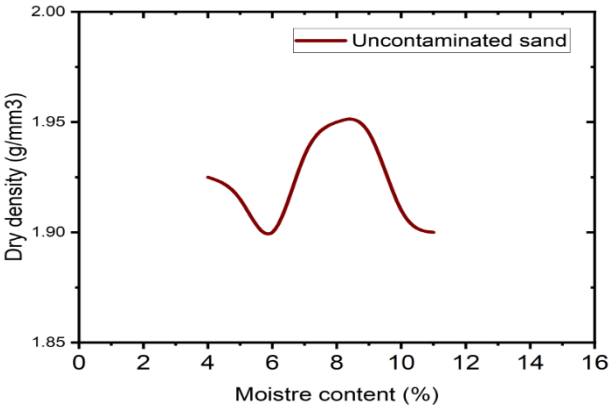


Figure 6. Compaction curve for uncontaminated sand

Figure 6 shows the compaction findings for an uncontaminated soil sample as a dry density against water content.The bulking impact of coastal sand is the reason for the early dip in the compaction curve.As CO concentration rises, **Figure 7** shows that both highest dry density and optimal water content typically decrease.The capillary action may be the cause of the drop in maximum dry density (Tang *et al.* 2012).The angle of contact and the medium's surface tension have an important impact on the capillariy tension. Crude oil keeps water from properly contacting soil particles because it is more hydrophobic than water. Thus, for samples contaminated with crude oil, lower values of maximum dry density arise from a drop in capillary tension as the crude oil concentration rises.CO's loss of compression energy may be another factor. Because crude oil is 40 times more viscous than water, increasing the tension between its molecules takes more compaction energy. Consequently, it takes more energy to raise the texture of the soil. The presence of CO in place of water may also lead to a reduction in the ideal water content, as it has the same effect as water (Taheri *et al.* 2018). **Figures 8 and 9** The maximum dry density and ideal moisture content of all oil percentages progressively increased for up to 30 days following mixing. After that, they gradually decreased since it took some time to mix thoroughly and reach equilibrium.

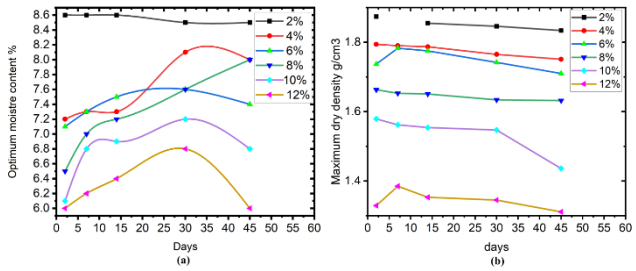


Figure 7. Oil contaminated sand: Relationship between optimum moisture content, maximum dry density and days for adding various percentage of oil in soil sample

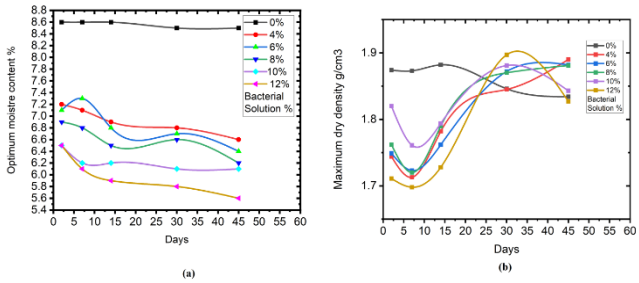


Figure 8. Biotreated sand: Relationship between optimum moisture content and days, maximum dry density and days for various percentage of bacterial solution added to 2% oil content soil sample

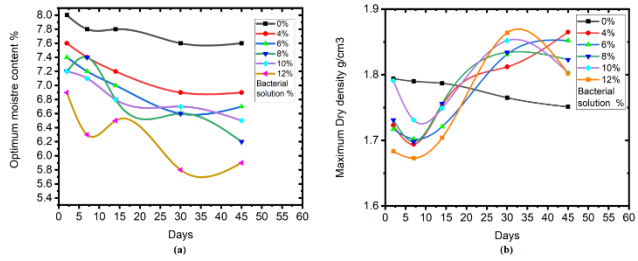


Figure 9. Biotreated sand: Relationship between optimum moisture content, maximum dry density and days for various percentage of bacterial solution added to 4% oil content soil sample

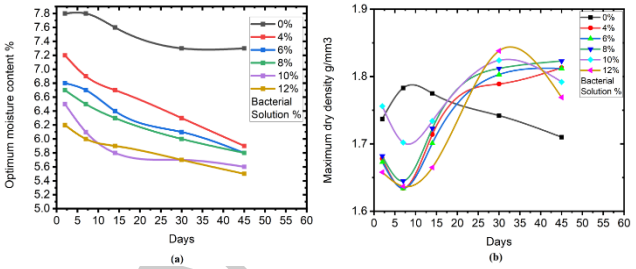


Figure 10. Biotreated sand: Relationship between optimum moisture content, maximum dry density and days for various percentage of bacterial solution added to 6% oil content soil sample

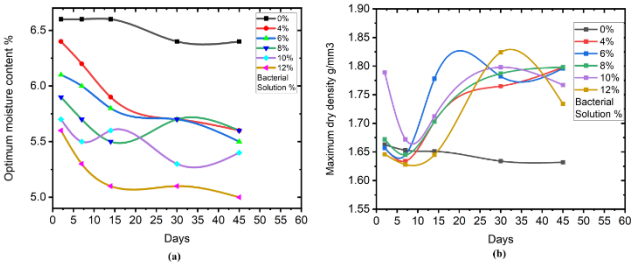


Figure 11. Bio treated sand: Relationship between optimum moisture content, maximum dry density and days for various percentage of bacterial solution added to 8% oil content soil sample

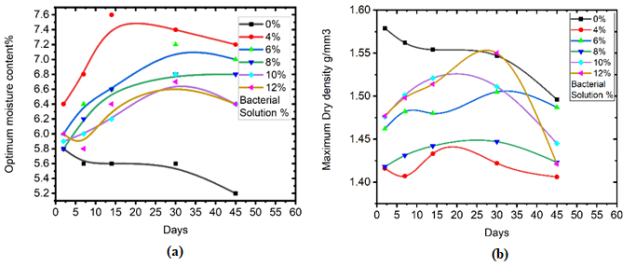


Figure 12. Biotreated sand Relationship between optimum moisture content and days for various percentage of bacterial solution added to 10% oil content soil sample.

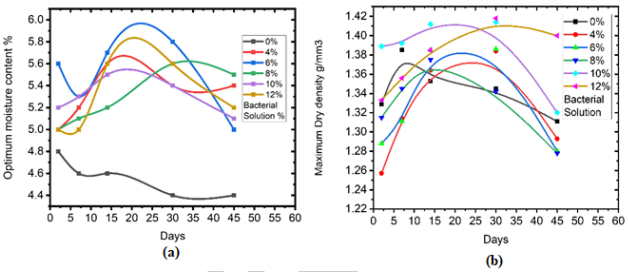


Figure 13. Biotreated sand Relationship between optimum moisture content, maximum dry density and days for various percentage of bacterial solution added to 12% oil content soil sample.

Figures 10 to 13 illustrate how biotreatment affects the ideal moisture content and the highest dry density. These numbers show that the maximum dry density rises and the ideal water content falls as bio-treatment increases. This tendency may have its roots in the physical characteristics of microbial biomass.Because the bacteria in microbial biomass are so tiny, their production fills the pore spaces between the soil particles, improving the compaction of bio-treated samples. (Dadashi *et al.* 2018). These numbers also show a tendency to increase the amount of bacterial solution from 2% to 10%, which results in a decrease in the optimal moisture content and a rise in maximum dry density. The optimum moisture content of virgin soil is 10%, hence up to 10 % oil contaminated sand uptakes bacterial solution after 10 % it cannot take much solution, hence the maximum dry density does not change much.

4.2. Direct shear test

Shear qualities are one of the most important aspects of any kind of soil. In accordance with *IS 2720 (Part 13): 1986*, the direct shear test samples were prepared and tested. Direct shear apparatus microprocessor based load 2kN capacity with proving ring and dial gauge AIMIL AIM 104-1. This characteristic is significant because it regulates the soil's bearing capacity and the foundation system's stability.Because the soil particles in every sample exhibit the strongest particle interaction at their respectivehighest dry density, the direct shear test samples were compacted to 0.95 times the maximum dry density value with the corresponding optimal water content.The impact of biotreatment and CO contamination on the soil's cohesiveness and internal friction angle is demonstrated in **Figures 14 to 18**.

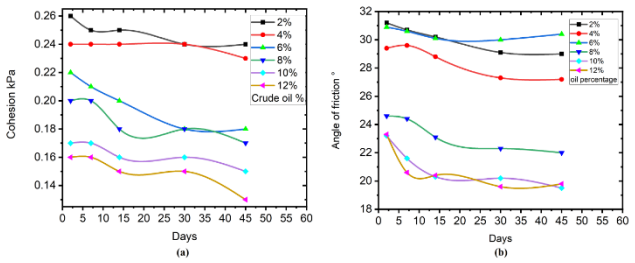


Figure 14. Oil contaminated sand: Relationship between Cohesion, Angle of friction and days for adding various percentage of oil in soil sample

The impact of CO pollution on the soil's cohesiveness and internal friction angle is displayed in **Figure 14**. In general, when the amount of CO in a sample increases, the values of cohesiveness and internal friction angle decrease. According to Shin *et al.* (2002) (Hemmat *et al.* 2010; Spiecker *et al.* 2023), oil contamination in sandy soils causes the internal friction angle to decrease, and Ghaly (2001) (Pourmohammadbagher and Shaw 2016) found that the internal friction angle decreases as the degree of oil contamination rises. The viscosity discrepancies between the water and crude oil may be the cause of this behaviour.The granular soil's shear strength decreases as the pore fluid's viscosity rises (Ogunbayo *et al.* 2021). Crude oil's lubricating properties also lessen inter-particle friction, which lowers the internal friction angle of the soil polluted by crude oil (Wu *et al.* 2020).

It is clear from **Figures 15 to 20** that when the original crude oil content of bio-treated samples increases, the internal friction angle reduces.The presence of microbial biomass in these samples affects the friction between particles.Microbial biomass is positioned between soil particles because it grows smaller than the soil particles.As a result, there is less surface contact between soil particles.Conversely, when a shear force is applied, the bacterial colonies tend to slide over one another, and those that do not resist are weaker than the soil particles.Consequently, there is less friction between soil particles (Kemper *et al.* 1984).

It is clear from the trend shown in **Figure 16** that as the percentage of bacteria and the duration of biotreatment increased, so did the samples' angle of internal friction. For instance, after 30 days of bacterial treatment of 6% original crude oil-contaminated sand, the angle of internal friction rose from 27.5° to 29.5° when the bacterial solution was raised from 4% to 12%, as shown in 4.12. Similarly, the angle of internal friction increased from 27.2° to 29.5° when 4% bacterial solution was added to4% initial crude oil-contaminated soil, which is shown in 4.11. It follows that the frictional resistance between the soil particles is increased when the sand sample is bioremediated using the microorganisms Bacillus amyloliquefaciens. The remediated sand sample's shear-strength properties improved as the/value increased.

Figure 16 shows that increased crude oil content causes low cohesion due to the viscosity and inherent cohesion of oil (Katukojwala *et al.* 2021). From **Figure 19, 20** we observed the trend that cohesion increased due to adding more bacterial solution. For example, in Figure 4.106% bacterial solution caused a 40% increase in cohesion when compared to the cohesion value of 2% initial oil-contaminated soil at 30 days biotreatment period. Additionally, the surface tension of pore fluid is a significant factor in soil cohesion (Bragg *et al.* 1994). Microbial biomass from these samples fills the pore spaces between particles and increases the surface area between particles, increasing the cohesion values in samples that have undergone biotreatment (Rathod *et al.* 2022). It may be deduced that the microbial biomass produces bio-surfactants, which raise the pore fluid's surface tension and improve soil cohesiveness (Hoff 1993; Tumeo *et al.* 1994).

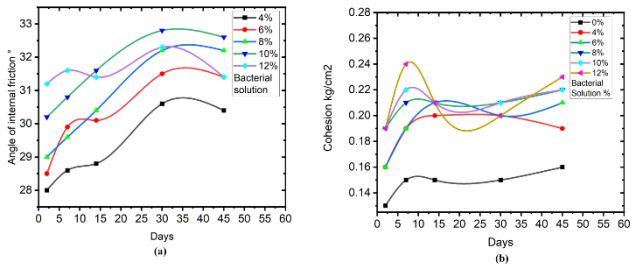


Figure 15. Biotreated sand Relationship between Angle of friction, cohesion and days, for various percentage of bacterial solution added to 2% oil content soil sample

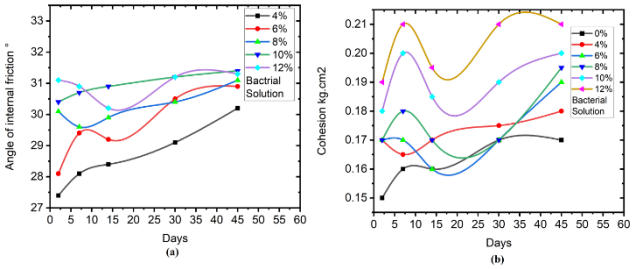


Figure 16. Bio treated sand Relationship between Cohesion, Angle of friction and days for various percentage of bacterial solution added to 4% oil content soil sample

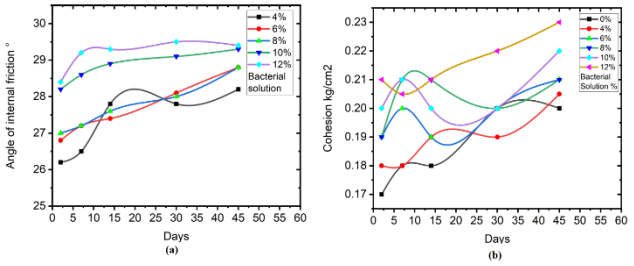


Figure 17. Biotreated sand Relationship between Angle of friction , cohesion and days for various percentage of bacterial solution added to 6% oil content soil sample

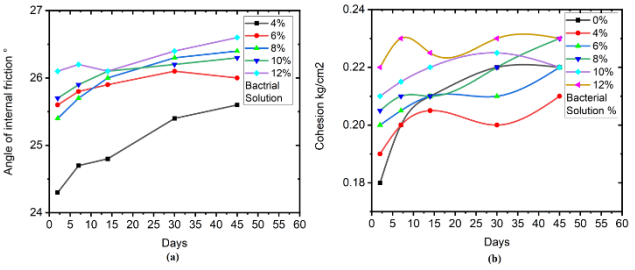


Figure 18. Bio treated sand Relationship between Cohesion , Angle of frictionand days for various percentage of bacterial solution added to 8% oil content soil sample

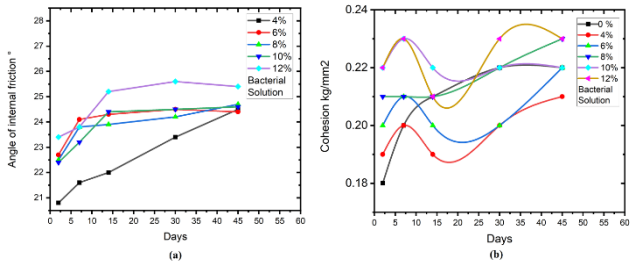


Figure 19. Biotreated sand Relationship between Angle of friction , Cohesion for various percentage of bacterial solution added to 10% oil content soil sample

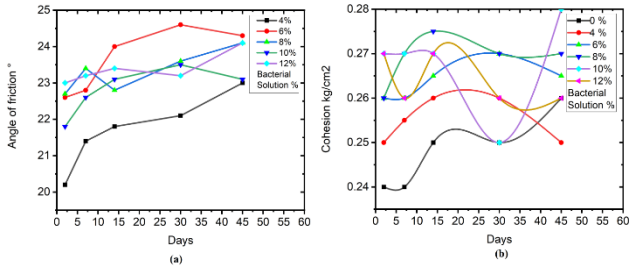


Figure 20. Biotreated sand: Relationship between Cohesion, Angle of friction and days for various percentage of bacterial solution added to 12% oil content soil sample.

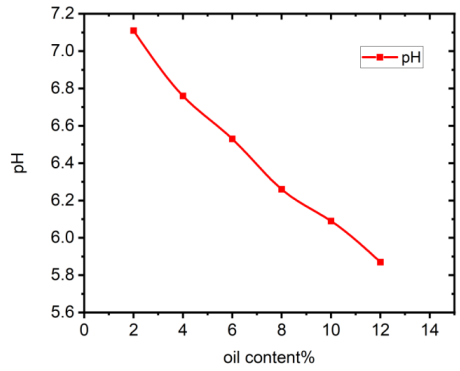


Figure 21. Oil contaminated sand: Relationship between ph and oil content 30 days curing period

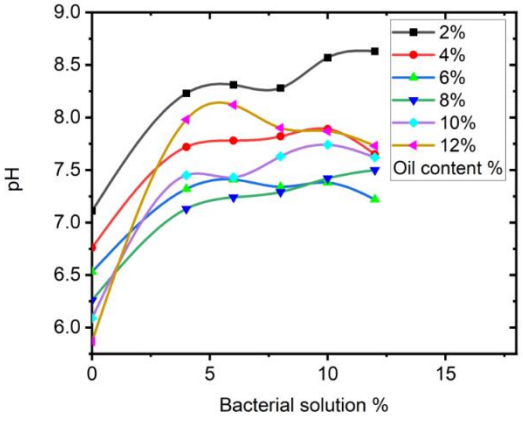


Figure 22. Biotreaed sand: Relationship between pH and bacterial solution for 10% oil contaminated Soil in 30 days biotreatment period

4.3. pH test

pH test was conducted on both various percentages of oil-contaminated sand in 30 days and various percentages of bioremediation sand in 30 days. In accordance with *IS 2720 (Part 13): 1986,* the procedure was followed for sample preparation and testing. Digital ph meter AIMIL 9815. From **Figure 21** it was discovered that adding the oil caused the soil's pH to drop. The heavy metals and chemicals found in crude oil are the cause of this decrease. There are trace levels of heavy metals, sulphur, and nitrogen in CO (Rahman *et al.* 2002; Singh *et al.* 2012; Hoffmann *et al.* 2016).The test results aligned with the findings of research by Tang *et al.* (2012), Fallah *et al.* (2015), and Taheri *et al.* (2018).

For bioremediation, sand the ph value of the sand increased with the addition of a higher percentage of bacterial solution. For example, **Figure 22** shows the ph level of 10% of oil-contaminated sand after adding various percentages of bacterial solution in 30 days. From this figure, pH value increased 21%,17%,13%19%,27%,34% for 2%,4%,6%,8%,10%,12% oil-contaminated sand when adding 10% bacterial solution in 30 days biotreatment time. The reason for the increment in ph value is that crude oil compounds are neutralized by bacterial biomass.

4.4. Chloride test

The presence of chlorinated hydrocarbons, which are often present in crude oil, caused the sample's chloride level to rise in the contaminated soil seen in **Figure 23**. Chloride determination was carried out in accordance with IS 2720 (Part 13): 1986, using a burette and pipette manufactured by Elico. The high chloride content in oil contaminated sand leads to corrosion of steel present in seashore structures.

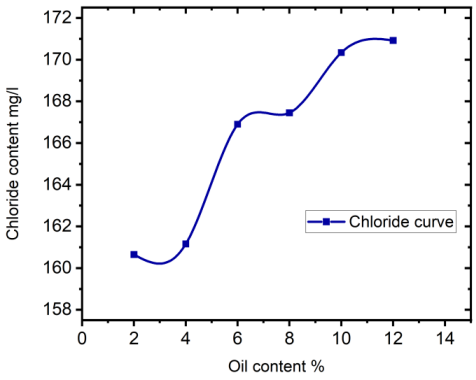


Figure 23. Oil contaminated sand relationship between chloride and oil content in 30 days curing period

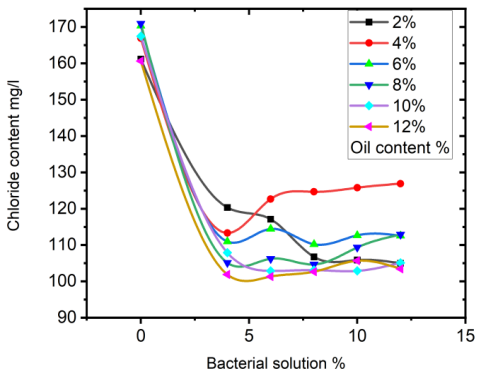


Figure 24: Biotreated sandrelationship between chloride andbacterial solution for 10% oil contaminated soil in 30days biotreatment period

A bacterial solution of various percentages was added to various percentages of oil-contaminated sand, and after 30 days of biotreatment time, the chloride content was measured. The results show chlorine values decrease after biotreatment. For example, in **Figure 24** chloride content was reduced by 53%,31%,51%,51%,59% 55% corresponding to 2%,4%,6%,8%,10%, and 12% oil contamination of sand remediated with 10% bacterial solution at biotreatment days of 30 days. It concludes the reduction of chlorinated hydrocarbons due to biotreatment.

4.5. Gravimetric analysis

Gravimetric analysis was done for various percentages of oil contamination and various percentages of bacterial solution for different biotreatment times. From the complete analysis result, it is concluded that crude oil concentration has reduced due to the degradation of bacterial biomass.

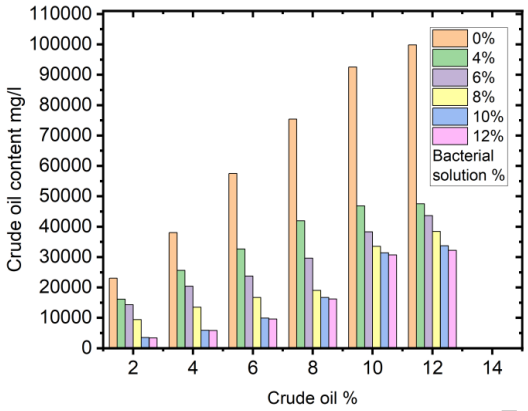


Figure 25. Biotreated sand: crude oil concentration of various bacterial concentration forvarious oil percentage at 30 daysbiotreatmenttime solution.

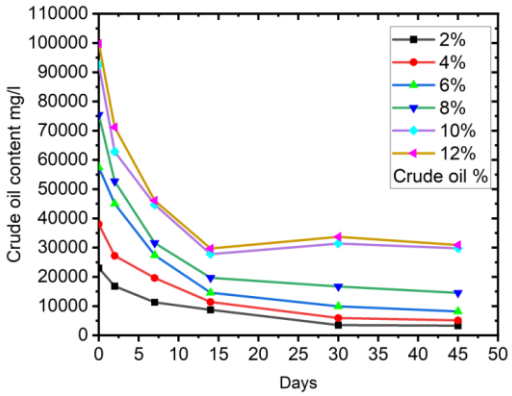


Figure 26. Biotreated sand: crude oil concentration of various oil percentage for different biotreatment time at 10% bacterial solution.

Figure 25 shows crude oil concentration in various percentages of biotreated samples versuscrude oil concentration at 30 days biotreatment. It indicates that bacterial concentration increase, crude oil concentration decrease effectively. For example 10% crude oil has initial crude oil concentration of 92,500 mg/kgdecreased to 46800 mg/kg, 38300mg/kg, 33500mg/kg, 31400mg/kg, 30700mg/kgcorresponding to adding 4%,6%,8%,10%,12% bacterial concentration respectively. **Figure 26** shows the concentration of CO in biotreated samples as a function of time (day).It makes sense that the crude oil concentration drops more noticeably as the bio-treatment time increases.For instance, after 2, 7, 14, 30, and 45 days of biotreatment, the crude oil concentration in bio-treated samples drops from the first CO content of 75,400 mg/kg soil to 52,700 mg/kg, 31,600 mg/kg, 19,700 mg/kg, 16,700, and 14,500 mg/kg soil, respectively.Because asphaltenes and other heavy components make up the majority of leftover CO, their limited solubility in water prevents it from leaching (Sharma *et al.* 2020; Colati *et al.* 2013). Furthermore, several studies have displayed that asphaltenes among soil particles enhance the sorption of organic molecules while not affecting water sorption (Wang *et al.* 2018). In actuality, the presence of different organic materials traps the leftover CO within soil particles; as a outcome, there is very little leaching of the leftover crude oil, making it non-toxic to the environment (Yu *et al.* 2021).

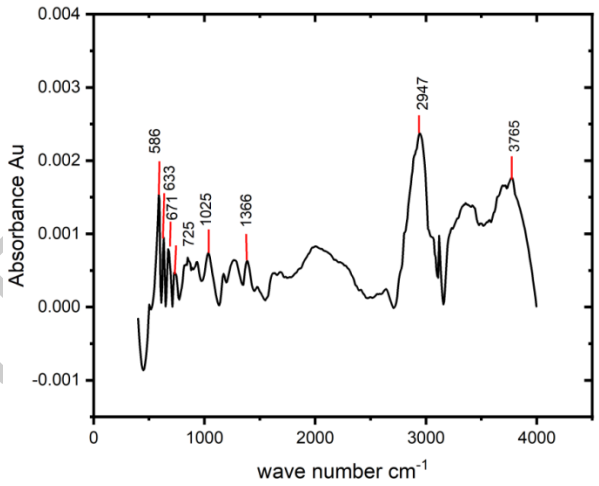


Figure 27. FTIR spectrum of 10% crude oil contaminated soil treated with 10 % bacterial solution after 30 days curing period.

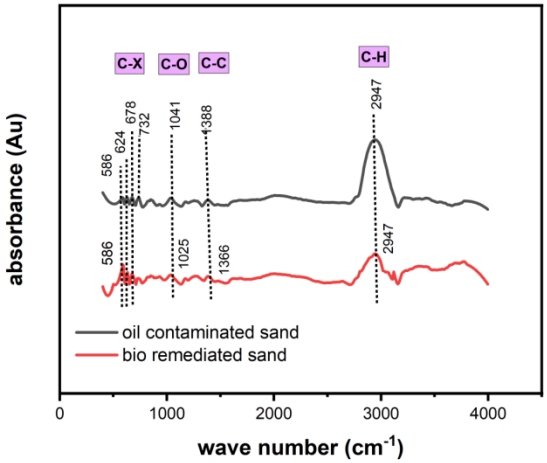


Figure 28. Comparative graph ofFTIR spectrum of oil contaminated and biotreated sand

4.6. FTIR

FT-IR technology, an important and practical technique for understanding surface functional groups and chemical binding behaviour, was performed using a SHIMADZU (Miracle 10) spectrometer.

The FTIR spectrum of a soil sample with 10% oil pollution that was treated with 10% bacterial solution and allowed to cure for 30 days is shown in **Figure 27**. **Table 3** lists the functional group details that correspond to the bond intensity and the observed discrete wave number band. Each peak's subareas were computed.

In comparison to the untreated sample, **Figure 28** shows how adding bacterial solution affects the biodegradation process. The C–C stretching vibration and the C–O stretching mode both move to lower wavelengths (from 1034 cm-1 to 1025 cm-1 and 1381 cm-1 to 1366 cm-1, respectively) in **Figure 8**. In contrast to soil polluted with CO, the strength of the C–H asymmetric 2947 cm-1 stretching vibrations decreased noticeably. Following bioremediation, the area under the peak of C-H stretching decreased from 1.107 units to 0.497 units. These changes showed that the bioremediation process had effectively removed crude oil from the soil.

Table 3. Characteristic infrared absorption frequencies present in the tested soil samples contaminated with engine oil

Soil+ crude oil			Soil+crudeoil+Bacteria		
Wavenumber (cm^{-1})	**Intensity**	**Peak assignment**	**Wavenumber (cm^{-1})**	**Intensity**	**Peak assignment**
3780	weak	O-Hstretch (Alcohols, Phenols)	3765	Weak	O-Hstretch (Alcohols, Phenols)
2947	Strong	C-H stretch (alkane)	2947	Strong	C-H stretch (alkane)
1381	Weak	C-C stretch	1366	Weak	C-C stretch
1034	Strong	C-O stretch(Alcohol)	1025	Strong	C-O stretch(Alcohol)
850-550	Strong	C-Cl stretch			
	850-550	Strong	C-Cl stretch		

The statistical analysis of the contaminated soil samples, as shown in **Table 4.**

Table 4. Statistical analysis of contaminated soil sample

Mean	17.6
Standard deviation	0.8
Upper limit	18.6
Lower limit	17.0

5. Discussion

The results indicate that crude oil contamination causes pronounced deterioration in the geotechnical and chemical characteristics of sand, as evidenced by reductions in MDD, OMC, cohesion, internal friction angle, and pH, along with an increase in chloride concentration. The geotechnical and chemical properties of sand, with MDD decreasing from 1.86 g/cm³ in uncontaminated sand to 1.61 g/cm³ in contaminated sand, OMC reducing from 12.4 % to 9.8 %, cohesion dropping from 18.2 kPa to 12.6 kPa, internal friction angle falling from 33.5° to 27.4°, and pH declining from 7.3 to 5.8, while chloride concentration increased from 42 mg/kg to 79 mg/kg. These negative impacts are due to the hydrophobic and viscous nature of crude oil, which coats soil particles, disrupts water retention, and reduces antiparticle bonding. In contrast, bioremediation with Bacillus amyloliquefaciens restored the MDD to 1.82 g/cm³, increased OMC to 12.1 %, improved cohesion to 17.5 kPa, raised the internal friction angle to 32.8°, and normalized pH to 7.1, while reducing chloride concentration to 45 mg/kg. This recovery is attributed to bacterial biomass filling soil pores, enhancing particle interlocking, and biosurfactant production that improved hydrocarbon breakdown and wettability. Gravimetric analysis confirmed a 68 % reduction in crude oil mass after treatment, and FTIR spectra showed marked attenuation of hydrocarbon-related peaks, confirming molecular-level degradation. Statistical analysis, with a mean value of 17.6 and a low standard deviation of 0.8, demonstrated high consistency and precision of measurements. Strong statistical foundation supports the validity of the observed effects of contamination and bioremediation, reinforcing the credibility of subsequent interpretations. Overall, the findings demonstrate that bioremediation not only recovers the mechanical stability and chemical quality of oil-contaminated sand but also provides a reliable and environmentally sustainable remediation method.

6. Conclusion

The impact of bio-treatment on marine sand polluted by crude oil that was gathered from the coastal region of Andhra Pradesh was examined in a comprehensive laboratory program. The soil sample was purposely polluted by the addition of CO, which increased from 2% by weight of dry samples to 12%. The contaminated soil samples were remediated by adding Bacillus amyloliquefaciens. Results were obtained for various biotreatment times and various bacterial concentrations. The studied materials and tests support the following conclusions:

- The optimum moisture content reduced by 6%, 9%, 13%, 16%, 18%, 26% for 2%, 4%, 6%, 8%, 10%, 12% of crude oil contaminated sand with 30 days mixing time when compared to virgin soil. Little up and down in optimum moisture content values when mixing time increases from 2 days to 30 days, then it does not vary much. This reduction in optimum moisture content was due to the effect of capillary tension and the presence of CO instead of water, which has the same effect as water.
- The Maximum dry density reduced by 6%, 11%, 13%, 20%, 21%, 45% for 2%,4%,6%,8%,10%,12% of crude oil contaminated sand with 30-days mixing time when compared to virgin soil. This may be due to the effect of capillary tension and wastage of compaction energy. Slight Reduction trend in maximum dry

density values when mixing time increases from 2 days to 30 days, then it does not get varied.

- The Angle of internal friction reduced by 14%, 21%, 9%, 48%, 63%,68%, for 2%,4%,6%,8%,10%,12% of crude oil contaminated sandwith 30 days mixing time when compared to virgin soil. This decrease results from the lubricating properties and increased viscosity of crude oil. Reduction of maximum dry densityvaluesof oil contaminated sand when mixing time increases from 2 days to 30 days then it does not vary much.
- Reduction of cohesion by 46%, 46%,94%, 94%, 118%, 133%, for 2%, 4%, 6%, 8%, 10%, 12% of crude oil contaminated sandwith 30 days mixing time when compared to virgin soil. This low cohesion due to viscosity and inherent cohesion of oil. Up to 30 days of mixing time cohesion reduced after that it does not vary much.
- Conclusion drawn from above results is 30 days of mixing time of oil with sand may be optimum.
- pH values of soil get reduced from 7.1 to 5.1 by adding oil from 2% to 12% at 30 days mixing period due to crude oil compounds.
- Virgin soil chloride content was 45mg/l and Chloride content of soil was increased from 160.8mg/l to 170.6mg/l due to adding oil from 2% to 12%. The reason for increment is chlorinated hydrocarbon in crude oil.
- Oil pollutants in the soil were better removed using the bacterial bioremediation approach.
- Adding bacterial concentration up to 10% raises the highest dry density andlowering the optimal moisture level in the biotreated sample, but little else changes.Increasing the biotreatment duration from two days to forty-five days increases the maximum dry density in all bacterial solutions.
- Angle of internal friction of soil gets reduced 40 to 50% by oil contamination when compared to virgin soil, after biotreatment this reduction gets improved around 70 to 80 %. This improvement due to microbial biomass. Similarly, cohesion also increased after biotreatment.
- Bacterial concentration up to 10 % gives good increment in angle of internal friction, more than 10% it increased slightly in more test and get reduced in some tests. But as the bio-treatment period lengthens, the angle of internal friction raises quickly for up to 30 days. After that, the increment slows but continues. The findings of the compaction and direct shear tests unequivocally demonstrate that biotreatment with a 10% bacterial concentration is required for more than 30 days in order for the bio-treated soil to perform better than the contaminated soil. Usually high bio treatment time need when using organic nutrient compared to chemical nutrient.
- Rate of degradation of crude oil concentration rapid increase up to 30 days bio treatmenttime and using 10% bacterial solution then that increment gets slow down. Bio treated soil At adding 10% bacterial solution with 30 days bio treatment time reduces crude oil concentrationof 85%, 84%, 83%, 78%, 66%, 66% when compared to oil contaminated sand of 2%, 4%, 6%, 8%, 10%, 12% respectively. This indicates Bacillus amyloliquefaciens gives better performance in mild and moderate contamination than in heavy contamination
- FTIR data show that, when applying 10% bacterial solution to 10% contaminated soil for 30 days of bio treatment; bioremediation reduces the oil concentration by 55% as compared to oil-contaminated soil.

The results obtained from a single microbial species and uniform soil type may not fully capture the complexity of real-world contaminated environments. This limitation may affect the generalizability of the findings across different soil textures, contaminant compositions, or microbial ecologies. Recognizing this, we have outlined plans for future studies involving multiple bacterial strains with varying metabolic capabilities and a broader range of soil types (e.g., clay, silt, and mixed sediments) to enhance the applicability and impact of the research outcomes in diverse geotechnical and environmental contexts. Long-term performance studies under field-scale conditions, inclusion of broader parameters such as enzymatic activity, heavy metal immobilization potential, and permeability enhancement. These directions are intended to expand the scope and translational value of the research and set the stage for future investigations that could yield more generalizable and high-impact outcomes.

Availability of supporting data

This paper does not generate or analyse any new data, hence it is not eligible for data sharing.

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