# CHAPTER I
# INTRODUCTION

## 1.1 Background

In many developing regions, the presence of subgrade soils with inadequate engineering properties poses a significant challenge to infrastructure development. Soils used as subgrade material may exhibit low bearing capacity and sensitivity to moisture variation, which can lead to excessive settlement, pavement deformation, cracking, and premature structural failure. These problems are particularly critical in road construction, where long-term pavement performance depends strongly on the strength and stability of the supporting soil layer.

To address these issues, soil stabilization techniques are commonly applied to improve subgrade performance. Conventional stabilization methods such as lime, cement, and fly ash have been widely used and proven effective in enhancing soil strength. However, these methods are often associated with high material costs, increased carbon emissions, and potential environmental impacts. Consequently, there is growing interest in alternative soil improvement techniques that are more sustainable and environmentally friendly.

One such alternative is biological soil stabilization through Microbially Induced Calcite Precipitation (MICP). This technique utilizes microbial activity to promote the formation of calcium carbonate ($CaCO_3$) within the soil matrix, which can improve particle bonding and reduce pore spaces. The presence of calcium carbonate may contribute to increased soil stiffness and strength, particularly when combined with appropriate compaction (Whiffin, 2004; DeJong et al., 2010).

Among various bacteria studied for MICP applications, *Bacillus tropicus* has attracted attention due to its adaptability to laboratory conditions and its potential to contribute to calcite precipitation. In this study, *Bacillus tropicus* was applied as a soil treatment material, and its effectiveness was evaluated based on the curing time of treated soil.

This research focuses on evaluating the effect of curing time on the California Bearing Ratio (CBR) of untreated and bacteria-treated soil under varying compaction energies (10, 25, and 56 blows). Both soaked and unsoaked conditions were considered to assess moisture sensitivity. By examining the combined effects of curing time and compaction energy, this study aims to provide insight into the short-term performance of biologically treated sand-dominated soil for subgrade applications.

## 1.2 Problem Statement

Weak subgrade soils with low bearing capacity remain a major concern in civil engineering projects, often requiring stabilization to meet design and performance requirements. Soil strength may decrease significantly under saturated conditions, making moisture sensitivity an important factor in subgrade evaluation. While various soil stabilization methods have been developed, the effectiveness of biological stabilization techniques depends strongly on curing time, compaction energy, and moisture condition.

In biological soil stabilization, curing time plays an important role in determining the development of soil strength after treatment. However, the short-term influence of curing time under different compaction energies and soaked versus unsoaked conditions has not been fully clarified, particularly for sand-dominated soils with plastic fines.

Therefore, this research investigates the effect of curing time on the California Bearing Ratio (CBR) of untreated and biologically treated soil using *Bacillus tropicus*. The influence of compaction energy and moisture condition is also examined to better understand their role in short-term soil strength development.

Based on this background, the research addresses the following questions:

a. What are the physical and mechanical properties of the soil used in this study?
b. How does curing time influence the CBR value of the soil under varying compaction energies and moisture conditions?
c. How do compaction energy and soaking condition affect the soil response during curing?
d. How do the CBR values of bacteria-treated soil compare with untreated soil after curing?

## 1.3 Research Objectives

The main objective of this research is to evaluate the effect of curing time on the California Bearing Ratio (CBR) of soil samples under different testing conditions.

The specific objectives of this study are to:

a. Determine the physical and mechanical characteristics of the soil used in this research.
b. Analyze the influence of curing time on CBR values under different compaction energies and moisture conditions.
c. Evaluate the effect of biological treatment using *Bacillus tropicus* on soil strength development.
d. Compare the CBR performance of untreated and biologically treated soils under soaked and unsoaked conditions.

## 1.4 Benefits of the Research

This research provides several contributions to the field of geotechnical engineering, particularly in sustainable soil stabilization:

- It provides experimental data on the short-term effect of curing time on CBR values of untreated and bacteria-treated soils.
- It offers insight into the influence of *Bacillus tropicus* treatment on soil strength development under limited curing duration.
- It clarifies the interaction between curing time, compaction energy, and moisture condition in subgrade performance.
- It contributes to ongoing research on environmentally friendly soil improvement techniques that may complement conventional stabilization methods.

## 1.5 Scope of the Research

To ensure that this study remains focused and systematic, the following scope and limitations were applied:

- This research was conducted as a laboratory-based experimental study at the Soil Mechanics Laboratory, Faculty of Engineering, Hasanuddin University.
- The soil used in this study was a natural soil classified as SP–SC (Poorly Graded Sand with Clay) according to the Unified Soil Classification System (USCS).
- Three soil conditions were examined: untreated soil, soil treated with *Bacillus tropicus* with a curing time of 0 days, and soil treated with *Bacillus tropicus* with a curing time of 4 days.
- Compaction was carried out using three compaction energy levels: 10, 25, and 56 blows per layer.
- California Bearing Ratio (CBR) tests were conducted under both soaked and unsoaked conditions.
- The primary engineering parameter evaluated in this study was the California Bearing Ratio (CBR) value.

## 1.6 Soil Types and Engineering Behavior

Soil is a naturally occurring material composed of mineral particles, water, and air, and it serves as the foundation for most civil engineering structures. Its engineering behavior directly influences the safety, performance, and durability of infrastructure.

In geotechnical engineering, soil behavior is commonly distinguished based on particle size distribution. Coarse-grained soils, such as sand and gravel, generally derive their strength from particle friction and interlocking, while fine-grained soils exhibit strength governed by cohesion and moisture sensitivity. However, when coarse-grained soils contain plastic fines, their engineering behavior may be significantly altered, particularly under saturated conditions.

The soil used in this study is classified as SP–SC, indicating a poorly graded sand containing clay fines. Although sand particles dominate the soil composition, the presence of plastic clay fines can reduce drainage capability and cause a noticeable decrease in strength when the soil is subjected to soaking. This behavior may result in lower CBR values under saturated conditions, limiting the suitability of the soil as subgrade material without improvement.

Therefore, this research focuses on evaluating the influence of curing time, biological treatment using *Bacillus tropicus*, and compaction energy on the CBR performance of SP–SC soil, with the aim of improving its short-term engineering behavior for subgrade applications.

## 1.7 Soil Classification

Soil classification provides a standardized system for grouping soils with similar engineering characteristics. In geotechnical engineering practice, soil classification is essential for predicting soil behavior under loading, moisture variation, and compaction, as well as for evaluating soil suitability for construction purposes.

According to Das (2010), soil classification does not aim to describe all mechanical properties of soil; instead, it groups soils with comparable behavior based on index properties such as particle size distribution and plasticity. Although natural soils are inherently variable, classification systems provide a practical framework for engineering analysis and preliminary design.

In this study, soil classification plays an important role because the research focuses on soil strength behavior, particularly the California Bearing Ratio (CBR), under different curing durations, compaction energy levels, and moisture conditions. Therefore, two internationally recognized soil classification systems were applied:

1. Unified Soil Classification System (USCS)
2. American Association of State Highway and Transportation Officials (AASHTO)

Both systems utilize particle size distribution and Atterberg limits to evaluate soil behavior. The results of these classifications form the basis for understanding the mechanical performance discussed in subsequent chapters.

### 1.7.1 Classification Based on Texture

Soil texture refers to the relative proportions of gravel, sand, silt, and clay particles within a soil mass. Texture plays a significant role in controlling permeability, plasticity, compressibility, and shear strength. Different institutions define soil particle size ranges slightly differently, as shown in Table 2.

Table 2. Particle Size Classification by Institution

| Institution | Gravel (mm) | Sand (mm) | Silt (mm) | Clay (mm) |
|---|---|---|---|---|
| MIT | >2 | 2–0.06 | 0.06–0.002 | <0.002 |
| USDA | >2 | 2–0.05 | 0.05–0.002 | <0.002 |
| AASHTO | 76.2–2 | 2–0.075 | 0.075–0.002 | <0.002 |
| USCS | 76.2–4.75 | 4.75–0.075 | <0.075 | — |

In many cases, natural soil contains a mixture of particles from several size ranges. Under texture-based classification, soil is named based on its dominant component, such as sandy clay or silty clay. The USDA classification system, for example, defines:

- Sand: particles between 2.0 mm and 0.05 mm
- Silt: particles between 0.05 mm and 0.002 mm
- Clay: particles smaller than 0.002 mm

The USDA triangle chart can be used to determine the texture classification of a soil sample based on its composition. For example, a soil sample with 30% sand, 40% silt, and 30% clay is categorized as clay loam.

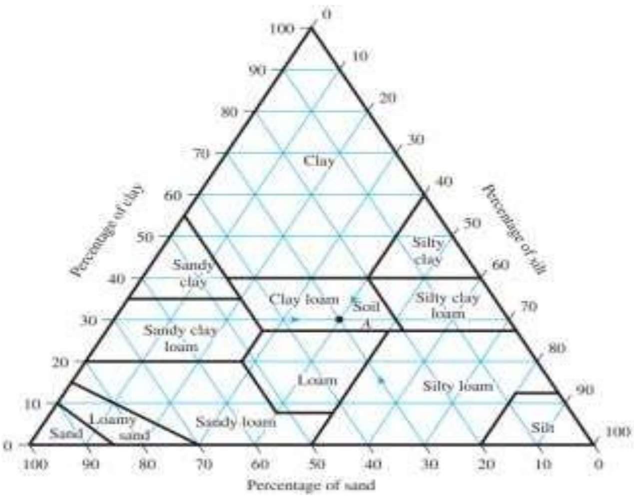


Figure 1. Classification according to USDA

Source : Das, Principles of Geotechnical Engineering 7th Edition (2010)

The use of the graph above is easiest to understand when shown with an example. If the particle size distribution of soil A shows 30% sand, 40% silt, and 30% clay, the textural classification can be determined by looking at the direction of the arrows shown in Figure 1. This soil falls within the clay loam zone. It should be noted that this graph is based only on soil that passes through a No. 10 sieve. Therefore, if the particle size distribution of a soil is such that a certain percentage of soil particles has a diameter greater than 2 mm, a correction needs to be made.

### 1.7.2 Classification Based on AASHTO

The AASHTO (American Association of State Highway and Transportation Officials) classification system was originally developed by Hogentogler and Terzaghi in 1929 and later updated in 1945. It was designed to evaluate soil suitability for use as a subgrade material in highway construction. This system remains widely used today due to its simplicity and its effectiveness in predicting soil behavior under road pavement.

AASHTO classifies soil into seven major groups, from A-1 to A-7, with further subgroups (e.g., A-1-a, A-2-4). Soils are grouped based on:

- Grain size distribution
- Atterberg limits (Liquid Limit and Plasticity Index)
- Percentage passing sieve No. 200 (0.075 mm)

Table 3. Grain Size Classification

| Soil Component | Sieve Size (mm) | Classification |
|---|---|---|
| Gravel | Retained on No. 10 (2.00 mm) | Coarse |
| Sand | Passes No. 10, retained on No. 200 | Intermediate |
| Silt and Clay | Passes No. 200 (0.075 mm) | Fine-grained (cohesive) |

### 1.7.3 AASHTO Classification Groups

- A-1 to A-3: Granular soils with ≤35% passing No. 200 sieve
- A-4 to A-7: Silty or clayey soils with >35% passing No. 200 sieve

Table 4. AASHTO Soil Classification Summary

| Group | % Passing No. 200 | LL | PI | Typical Soil Type | Subgrade Rating |
|---|---|---|---|---|---|
| A-1-a | ≤ 35% | ≤ 40 | ≤ 6 | Well-graded gravel and sand | Excellent to Good |
| A-2-4 | ≤ 35% | ≤ 40 | ≤ 10 | Silty gravel or silty sand | Good to Fair |
| A-4 | > 35% | ≤ 40 | ≤ 10 | Non-plastic silt | Fair to Poor |
| A-6 | > 35% | ≤ 40 | > 10 | Clayey soil (low plasticity) | Poor |
| A-7-6 | > 35% | > 40 | > 10 | High plasticity clay | Very Poor |

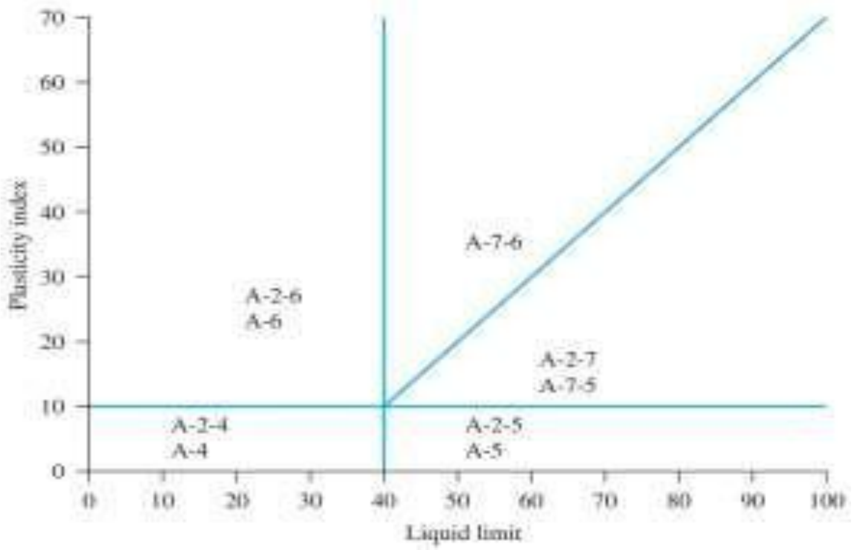


Figure 2. Range of liquid limit and plasticity index values in groupsA-2, A-4, A-5, A-6, dan A-7, *Source: Das, Principles of Geotechnical Engineering 7th Edition (2010)*

After analyzing a soil sample's liquid limit (LL) and plasticity index (PI), its position on the AASHTO plasticity chart (Figure 2) helps determine the proper classification group.

The area above the "A-line" typically represents clayey soils (A-6 and A-7-6), while the area below the line indicates silty soils (A-4, A-5, and A-7-5). The slope of the line helps distinguish between plastic and non-plastic behavior, with higher values of LL and PI indicating higher plasticity and swelling potential.

For example:

- Soils falling in the A-4 zone generally have low plasticity and perform moderately well as subgrade.
- Soils in the A-6 group have plastic clayey fines, showing greater sensitivity to moisture changes.
- Soils classified under A-2-6 typically exhibit high plasticity and poor engineering behavior, including high shrink-swell potential and low strength, especially when wet.

In this Study:

The soil contained 9.6% passing the No. 200 sieve, with a liquid limit of 52% and a plasticity index of 19%. Based on these values, the soil is classified as A-2-7, indicating the soil in this study is a coarse-grained soil dominated by sand with plastic fines.

### 1.7.4 Soil Classification Based on USCS (Unified Soil Classification System)

The Unified Soil Classification System (USCS) was originally developed by Professor A. Casagrande in 1948 for use in airfield construction during World War II. It was later modified in 1952 by Casagrande, the U.S. Bureau of Reclamation, and the U.S. Army Corps of Engineers to make it applicable for dams, foundations, and other major civil works.

The USCS classifies coarse-grained soils based on grain size distribution, while the classification of fine-grained soils relies primarily on plasticity behavior, determined through Atterberg limits. In other words:

- If the soil's behavior is controlled by coarse particles (sand or gravel), it is classified by gradation.
- If the behavior is controlled by fines (silt or clay), it is classified by plasticity.

To apply USCS classification, only sieve analysis and Atterberg limit tests are required (Holtz and Kovacs, 1981).

### 1.7.5 USCS Soil Categories

USCS divides soil into two major categories:

**a.** Coarse-grained soils:

- Less than 50% passing the No. 200 sieve (0.075 mm).
- Group symbols begin with:
  a) G: Gravel
  b) S: Sand

**b.** Fine-grained soils:

- 50% or more passing the No. 200 sieve.
- Group symbols begin with:
  a) M: Inorganic silt
  b) C: Inorganic clay
  c) O: Organic silt or clay
  d) Pt: Peat or highly organic soils

### 1.7.6 Additional Group Symbols

- $W$ = Well graded
- $P$ = Poorly graded
- $L$ = Low plasticity (LL < 50)
- $H$ = High plasticity (LL ≥ 50)

Table 5. Unified Soil Classification System (USCS)

| Soil Type | Criteria | Symbol |
|---|---|---|
| Gravel (clean) | Cu ≥ 4, 1 ≤ Cc ≤ 3 | GW |
| | Cu < 4 and/or Cc < 1 or > 3 | GP |
| Gravel with fines | PI < 4 or plots below A-line | GM |
| | PI > 7 and plots on or above A-line | GC |
| Sand (clean) | Cu ≥ 6, 1 ≤ Cc ≤ 3 | SW |
| | Cu < 6 and/or Cc < 1 or > 3 | SP |
| Sand with fines | PI < 4 or plots below A-line | SM |
| | PI > 7 and plots on or above A-line | SC |
| Silt or Clay (LL < 50) | PI < 4 or below A-line | ML |
| | PI > 7 and above A-line | CL |
| Silt or Clay (LL ≥ 50) | PI < 4 or below A-line | MH |
| | PI > 7 and above A-line | CH |
| Organic soils | LL(oven dried) / LL(not dried) < 0.75 | OL, OH |
| Peat | Highly organic, fibrous | Pt |

$Cu = D_{60} / D_{10}$
$Cc = (D_{30})^2 / (D_{10} \times D_{60})$

### 1.7.7 Plasticity Chart

The classification of fine-grained soils (M and C groups) uses a Plasticity Chart (Figure 3) that plots Plasticity Index (PI) against Liquid Limit (LL).

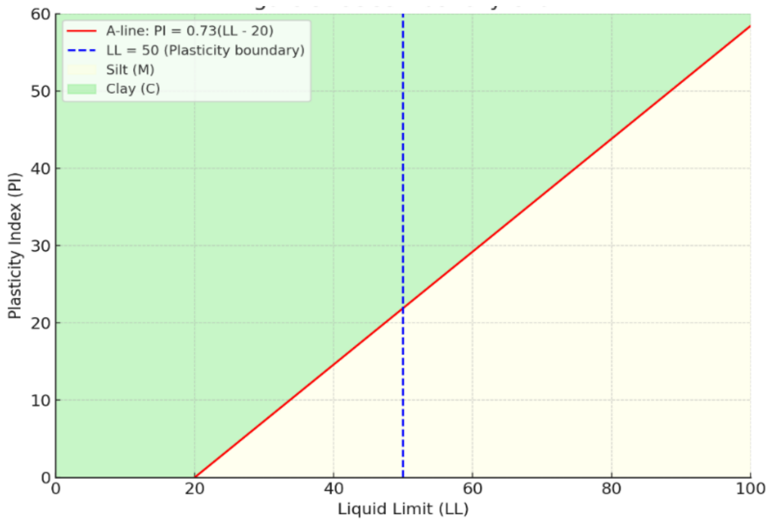


Figure 3. USCS plasticity chart

- Soils above the A-line = Clay (C)
- Soils below the A-line = Silt (M)
- LL < 50 → Low plasticity (L); LL ≥ 50 → High plasticity (H)

Data Required for USCS Classification

To classify a soil using USCS, the following data are typically needed:

1. Gravel content = % retained on No. 4 (4.75 mm)
2. Sand content = % passing No. 4 and retained on No. 200
3. Fines content = % passing No. 200
4. Cu and Cc = Coefficients of uniformity and curvature
5. Atterberg limits = LL and PI from fine fraction (passing No. 40)

The soil group symbol is assigned based on this information. Dual symbols (e.g., GW-GM, SP-SM) are used when a soil has 5–12% fines.

### 1.7.8 Application in This Study

The soil sample used in this research had:

- 9.6% passing the No. 200 sieve
- LL = 52%, PI = 19%

Based on the grain size distribution results, the soil is dominated by sand-sized particles, with a relatively small percentage of fines. Since less than 50% of the soil passes the No. 200 sieve, the soil is classified as a coarse-grained soil according to the Unified Soil Classification System (USCS).

The fines content falls within the range of 5–12%, which requires the use of a dual symbol in USCS classification. Evaluation of the Atterberg limits indicates that the fines are plastic and clayey in nature, as the Plasticity Index (PI) plots above the A-line on the plasticity chart and exceeds 7%.

In addition, the gradation analysis shows that the sand fraction is poorly graded, as indicated by a coefficient of uniformity (Cu) less than 6. Therefore, based on gradation characteristics and plasticity behavior of the fines, the soil is classified as:

SP–SC (Poorly Graded Sand with Clay)

This classification indicates that although the soil has a predominantly sandy structure with relatively good drainage characteristics, the presence of plastic clay fines significantly influences its engineering behavior. These fines contribute to increased moisture sensitivity, reduced strength under soaked conditions, and variability in bearing capacity.

As a result, the soil in its natural condition is not ideal for use as subgrade material, particularly when exposed to water. This condition justifies the need for stabilization. In this study, biological stabilization using *Bacillus tropicus* is applied to evaluate its effectiveness in improving the California Bearing Ratio (CBR) performance of SP–SC soil under different curing durations and compaction energy levels.

## 1.7.9 Physical Properties of Soil

The physical properties of subgrade soil play a fundamental role in determining its engineering behavior and overall performance. These properties control how soil responds to loading, compaction, and variations in moisture content. According to Kusuma et al. (2016), physical properties are intrinsic characteristics related to the composition and internal structure of soil and significantly influence strength, compressibility, and permeability.

In geotechnical engineering practice, physical properties are commonly expressed using index parameters such as moisture content, specific gravity, void ratio, porosity, degree of saturation, and Atterberg limits. These parameters form the basis for soil classification and provide essential input for interpreting mechanical test results such as compaction and California Bearing Ratio (CBR). In this study, the evaluation of physical properties establishes a fundamental understanding of the soil behavior prior to biological treatment and curing.

### 1.7.9.1 Moisture Content (Water Content)

Moisture content is the ratio of the weight of water ($W_w$) to the weight of solid particles ($W_s$) in a soil sample, expressed as a percentage:

$$\omega = \frac{W_w}{W_s} \times 100\%$$

Where :

$\omega$ = moisture conent

$W_w$ = weight of water

$W_s$ = weight of dry soil

Moisture content significantly affects soil compaction, strength, and deformation characteristics.

### 1.7.9.2 Specific Gravity (Gs)

Specific gravity is the ratio of the unit weight of solid particles ($\gamma_s$) to the unit weight of water ($\gamma_w$) at 4°C:

$$G_s = \frac{\gamma_s}{\gamma_w}$$

where:

$Gs$ = specific gravity

$\gamma s$ = unit weight of soil solids

$\gamma w$ = unit weight of water

Table 6. Typical Specific Gravity Values for Different Soil Types

| Type of Soil | Spesific Gravity (Gs) |
|---|---|
| Gravel | 2.65 - 2.68 |
| Sand | 2.65 - 2.68 |
| Inorganic Soil | 2.62 - 2.68 |
| Organic Clay | 2.58 - 2.65 |
| Inorganic Clay | 2.68 - 2.75 |
| Humus | 1.37 |
| Peat | 1.25 - 1.80 |

Source: Hardiyatmo, Soil Mechanics I (2002)

#### 1.7.9.3 Void Ratio (e)

Void ratio is the ratio between the volume of voids ($V_v$) and the volume of solids ($V_s$). Higher void ratio generally indicates lower soil strength:

$$e = \frac{V_v}{V_s}$$

where:
$e$ = void ratio
$Vv$ = volume of voids
$Vs$ = volume of solids

A higher void ratio generally indicates lower soil density and reduced strength.

#### 1.7.9.4 Porosity (n)

Porosity is the ratio between the volume of voids ($V_v$) and the total volume of soil ($V$). It is usually expressed as a percentage:

$$n = \frac{V_v}{V}$$

where:
$n$ = porosity
$Vv$ = volume of voids
$V$ = volume of soil

Porosity is usually expressed as a percentage and reflects the soil's capacity to hold air and water.

#### 1.7.9.5 Degree of Saturation (S)

The degree of saturation is the ratio of the volume of water ($V_w$) to the volume of voids ($V_v$). This indicates how much of the pore space is filled with water:

$$S = \frac{V_w}{V_v} \times 100\%$$

where:
$S$ = degree of saturation
$V_w$ = volume of water
$V_v$ = volume of voids

Table 7. Soil Consistency Based on Degree of Saturation

| Soil Consistency | Degree of Saturation (S) |
|---|---|
| Dry Soil | 0.00 |
| Slightly damp soil | >0 – 0.25 |
| Moist soil | 0.26 – 0.50 |
| Very moist soil | 0.51 – 0.75 |
| Wet soil | 0.76 – 0.99 |
| Saturated soil | 1.00 |

Source: Hardiyatmo, Soil Mechanics I (2002)

### 1.7.10 Mechanical Properties of Soil

Mechanical properties describe the behavior of soil when subjected to external loads or stresses. According to Rochmawati and Irianto (2020), these properties are critical in geotechnical engineering because they directly influence soil strength, deformation, and stability. Mechanical properties are commonly evaluated through laboratory tests such as compaction tests and the California Bearing Ratio (CBR) test.

#### 1.7.10.1 Soil Compaction

Soil compaction is a mechanical process that reduces air voids within the soil mass and increases its density through particle rearrangement without altering grain size. Compaction improves shear strength and bearing capacity while reducing compressibility and permeability.
In engineering practice, compacted soil is widely applied as:

- Subgrade material for pavements
- Foundation base layers
- Earth dam and embankment material
- Backfill behind retaining walls
- Levees and roadway embankments

When soil in its natural condition exhibits low strength or excessive deformation, improvement is required before use. Compaction is one of the most practical and economical soil improvement methods, especially for soils intended for foundation and pavement layers.

The following formulas are used in laboratory compaction tests:

1) Wet (bulk) Unit Weight ($\gamma_{wet}$)

$$\gamma_{wet} = \frac{W_{total}}{V_t}$$

2) Dry Unit Weight ($\gamma_{dry}$)

$$\gamma_{dry} = \frac{\gamma_{wet}}{(1 + \boldsymbol{\omega})}$$

3) Total Unit Weight ($\gamma$)

$$\gamma = \frac{W_t}{V_t} = (\gamma_d)\left(1 + \frac{\boldsymbol{\omega}}{100}\right)$$

where:

$\gamma_{wet}$ = wet unit weight

$\gamma_{dry}$ = dry unit weight

$W_{total}$ = total weight of soil

$V$ = volume of soil

$\boldsymbol{\omega}$ = moisture content (decimal)

As water content increases during compaction, the dry unit weight initially rises due to improved particle lubrication and rearrangement. The dry unit weight reaches a maximum at the optimum moisture content (OMC). Beyond this point, excess water occupies pore spaces and causes a reduction in dry density. At zero moisture content, the wet and dry unit weights are equal.

**1.7.10.2 California Bearing Ratio (CBR) Test**

The California Bearing Ratio (CBR) test is a laboratory method used to evaluate the bearing capacity of subgrade, subbase, and base materials. It is widely applied in pavement and highway design to assess soil load-supporting capability. The test was developed by the California Division of Highways and remains a standard procedure in geotechnical engineering.

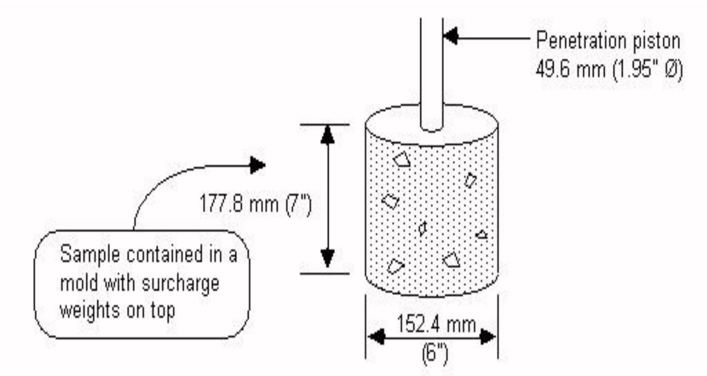


Figure 4. Schematic Diagram of the California Bearing Ratio (CBR) Test Setup

Figure 4 illustrates the standard CBR test setup. A cylindrical soil specimen is compacted inside a CBR mold with an internal diameter of 152.4 mm and a height of 177.8 mm. A penetration piston with a diameter of 49.6 mm applies a vertical load at a constant penetration rate of 1.25 mm/min. The resistance offered by the soil against penetration is recorded and used to determine the CBR value.

This configuration represents the testing method used in this study to evaluate the effect of bacterial treatment and curing time on soil strength.

**1) Objective and Application**

The primary objective of the CBR test is to assess the relative strength of compacted soil or granular materials by comparing them to a standard crushed stone. The test results are used to:

- Design pavement thickness
- Evaluate the effectiveness of soil stabilization
- Determine the impact of moisture and curing time on soil strength
- Compare the performance of treated vs. untreated soil

In this study, the CBR test is the main method used to assess the improvement of soil treated with *Bacillus tropicus* bacteria.

**2) Test Procedure Overview**

1. Sample Compaction: Soil samples are compacted in molds using three different compaction energies (10, 25, and 56 blows per layer) to simulate various field conditions.
2. Soaking (for soaked samples): Some samples are submerged in water for four days to simulate saturated subgrade conditions.
3. Penetration Test: A plunger with a diameter of 50 mm penetrates the compacted soil at a constant rate of 1.25 mm/min.
4. Load Measurement: Loads are recorded at standard penetration depths of 2.5 mm and 5.0 mm.

**3) CBR Formula**

$$CBR = \left(\frac{P}{P_s}\right) \times 100\%$$

where:
$P$ = measured load at a specific penetration (kg or N)
$P_s$ = standard load

- 1370 kg for 2.5 mm
- 2055 kg for 5.0 mm

The higher value between 2.5 mm and 5.0 mm penetration is typically used as the final CBR result.

**4) Relevance to This Research**

This study evaluates the influence of curing time on CBR values using *Bacillus tropicus.* Tests were conducted on:

- Untreated (natural) soil
- Soil treated with 0-day curing bacteria
- Soil treated with 4-day curing bacteria

Each condition was tested under soaked and unsoaked states with varying compaction energies. The analysis focused on:

- The effectiveness of bacterial treatment
- The role of curing time
- Improvements in soil bearing capacity

The CBR test provides a practical and field-representative method for assessing soil improvement achieved through environmentally friendly biological stabilization.

### *1.7.10.3 Bacillus tropicus*

*Bacillus tropicus* is a Gram-positive bacterium belonging to the genus Bacillus, commonly found in soil and natural environments. Bacteria within this genus are known for their resilience and ability to produce enzymes such as protease, lipase, amylase, and cellulase, making them suitable for biotechnological and geotechnical applications.

Mahawish et al. (2018) reported that Bacillus species function as decomposer bacteria capable of breaking down complex organic compounds. Jeong et al. (2017) further identified these bacteria as denitrifying and ammonifying organisms involved in nitrogen cycling.

**a) Mechanism of Soil Stabilization**

In soil stabilization, *Bacillus tropicus* contributes through ureolytic activity. Urease enzymes hydrolyze urea into ammonia ($NH_3$) and carbon dioxide ($CO_2$), increasing the pH of the surrounding environment. In the presence of calcium ions ($Ca^{2+}$), this process leads to calcium carbonate ($CaCO_3$) precipitation.

The precipitated $CaCO_3$ crystals bind soil particles together, improving interparticle bonding and increasing soil strength and stiffness. This process is classified as microbial-induced calcite precipitation (MICP), a biogrouting technique that enhances soil properties at the pore scale with minimal environmental impact (De Muynck et al., 2010; Al Qabany et al., 2012).

**b) Taxonomy of *Bacillus tropicus***

- Kingdom: Bacteria
- Phylum: Firmicutes
- Class: Bacilli
- Order: Bacillales
- Family: Bacillaceae
- Genus: Bacillus
- Species: *Bacillus tropicus*

**c) Calcite Precipitation Process**

According to Gusmawati et al. (2009), calcium ions accumulate on bacterial cell surfaces, forming nucleation sites for $CaCO_3$ crystal growth. Mukherjee et al. (2019) stated that calcite precipitation efficiency depends on bacterial concentration, pH, ionic strength, and curing duration.

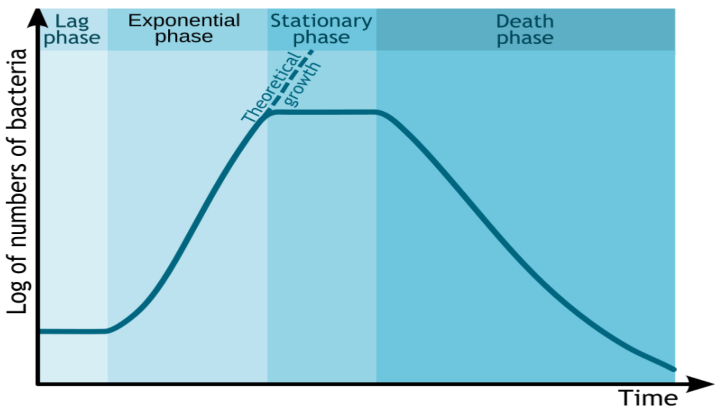


Figure 5. Bacterial Growth Curve

***Source**: Michał Komorniczak, Wikimedia Commons (2009). Licensed under CC BY-SA*

*3.0*

Bacterial growth is typically represented by a growth curve obtained through turbidity monitoring in liquid culture media. The growth curve consists of four main phases (Sharma et al., 2021):

- Lag phase
- Exponential phase
- Stationary phase
- Decline phase

Understanding these growth phases is important for determining the appropriate bacterial age and helps to evaluate the appropriate curing time for soil treated with different bacterial ages to help understand the relationship between the bacterial age and the curing time and how to optimize the soil strength using both the growth of the bacteria and the curing time which is the main use in this study.

## 1.8 Previous Research And Research Gap and Relevance to This Study

Numerous studies have investigated microbial-based soil stabilization using Bacillus species to improve the engineering properties of weak soils. Research indicates that Bacillus subtilis, Bacillus pasteurii, Bacillus megaterium, and *Bacillus tropicus* are capable of enhancing soil strength through MICP by promoting calcium carbonate precipitation.

Previous studies generally report that stabilization effectiveness depends on bacterial type, concentration, curing time, soil type, and moisture condition. However, most existing research focuses on curing periods of seven days or longer.

Limited studies have examined short-term curing durations, particularly curing periods of four days or less, which are highly relevant for rapid construction and temporary road applications. In addition, research on *Bacillus tropicus*, especially regarding the influence of the soil curing time, remains limited. Few studies have systematically evaluated the combined effects of bacterial age, compaction energy, and soaking condition on CBR performance.

Therefore, this study addresses these gaps by investigating the effect of curing time on CBR values using *Bacillus tropicus* with 0-day and 4-day curing conditions under varying compaction energies and soaked–unsoaked states. The findings are expected to provide practical insight into the feasibility of short-term, environmentally friendly soil stabilization for subgrade improvement.

Table 8. Summary of Previous Studies Related to Soil Stabilization Using Bacteria

| Author(s) | Title | Objectives and Key Findings | Source |
|---|---|---|---|
| R. Ghasemi & A. Jafarzadeh (2018) | *CBR Improvement in Sandy Soil Using Microbial Calcium Carbonate Precipitation* | Investigated sandy soil treated using MICP with *Bacillus pasteurii*. The study reported that CBR values increased with higher bacterial concentration and longer curing time. Optimum improvement was achieved using 6% bacterial content with a curing period of 7 days. | *Geomicrobiology Journal* |
| S. Satyamurthy & K. Sharma (2016) | *Strength Improvement of Soil Using Microbial Technique* | Examined clayey soil stabilized using *Bacillus subtilis* through MICP. Results showed that after 7 days of curing, CBR values increased by nearly three times compared to untreated soil, indicating significant strength enhancement. | *International Journal of Innovative Research in Science, Engineering and Technology* |
| R. Hamdan, A. Basri & N. Fauzi (2021) | *Application of Microbial Treatment to Improve Soil Bearing Capacity* | Evaluated soil bearing capacity improvement using *Bacillus megaterium*. The study reported notable increases in CBR values, particularly under soaked conditions after 4 days of curing, indicating improved water resistance and load-bearing performance. | *Journal of Civil Engineering Frontiers* |
| T. A. Nafiah, I. R. Ridwan & M. F. Adam (2020) | *Effect of Bacteria on Subgrade Soil Stabilization Using MICP* | Investigated silty–clay subgrade soil treated with *Bacillus subtilis* at various curing periods. The results showed up to a 200% increase in CBR values after 7 days of curing compared to untreated soil. | *Proceedings of National Seminar on Infrastructure Technology (SENTRA)* |
| DeJong et al. (2013) | *Biocementation in Road Subgrade Layers Using MICP* | Studied microbial treatment of road subgrade layers using several *Bacillus* species. The findings confirmed that bacterial treatment combined with a calcium source significantly improved CBR values, with effectiveness strongly influenced by bacterial concentration and curing duration. | *Transportation Geotechnics Journal* |

# CHAPTER II
# METHODOLOGY

## 2.1 Time and Location of Research

This research was conducted as an experimental laboratory study using natural soil samples obtained for laboratory investigation. The soil was selected based on visual characteristics and preliminary field observations, indicating a natural subgrade material containing a significant proportion of sand with plastic fines. Such soil types are commonly encountered in pavement and foundation applications.

The detailed classification of the soil was determined through laboratory testing, including grain size distribution and Atterberg limits, in accordance with the Unified Soil Classification System (USCS) and the AASHTO soil classification system. Soils of this type generally exhibit moderate bearing capacity reduction, sensitivity to moisture variation, and strength degradation under saturated conditions. Therefore, soil improvement was considered necessary to enhance its mechanical behavior and suitability for use as subgrade material.

The main objective of this research was to evaluate the effect of curing time on the California Bearing Ratio (CBR) value of soil, with particular emphasis on short-term curing performance. To achieve this objective, both untreated and biologically treated soil samples were tested. Biological treatment was conducted using *Bacillus tropicus* bacteria to improve soil properties through the mechanism of Microbially Induced Calcite Precipitation (MICP).


Figure 7. Location of soil test

All laboratory testing activities were carried out at the Soil Mechanics Laboratory, Department of Civil Engineering, Faculty of Engineering, Hasanuddin University, Gowa.

This study specifically examined the influence of curing duration on soil bearing capacity as evaluated using the CBR test. The research involved the following sample categories:

a. Untreated soil (control sample)
b. Soil treated with *Bacillus tropicus* - curing time 0 days
c. Soil treated with *Bacillus tropicus* - curing time 4 days

Each sample category was tested under both soaked and unsoaked conditions using three different compaction energy levels, namely 10, 25, and 56 blows per layer.

The research was conducted within the following time frame:

- Soil sampling, preparation, bacterial treatment, and laboratory testing: July 2024 – September 2024

## 2.2 Data Collection Method

Data collection in this research was carried out through a series of controlled laboratory tests. The process began with the selection and preparation of natural soil samples, followed by systematic testing to determine their physical and mechanical properties.

The laboratory testing program was divided into two main stages, as described below.

### 2.2.1 Physical Properties Testing

To understand the basic characteristics of the soil, the following tests were conducted:

- Moisture content
- Specific gravity
- Grain size distribution (sieve analysis)

### 2.2.2 Mechanical Properties Testing

To evaluate the engineering behavior of the soil under different treatments, the following tests were performed:

- Standard Proctor Compaction Test (to determine optimum moisture content and maximum dry density)
- California Bearing Ratio (CBR) Test (performed under soaked and unsoaked conditions)

The CBR test was used as the primary method to evaluate the impact of *Bacillus tropicus* on soil bearing capacity. Comparisons were made between:

- Untreated soil.
- Soil treated with *Bacillus tropicus* - curing time 0 days.
- Soil treated with *Bacillus tropicus* - curing time 4 days.

All treated samples were subjected to a curing period of 4 days prior to testing. For soaked specimens, samples were submerged in water for four days to simulate saturated subgrade conditions, during which swelling readings were recorded daily using a dial gauge. It should be noted that this soaking period is a standard CBR testing protocol and is distinct from the curing period; submersion in plain water does not constitute curing, as it does not provide the nutrient-rich conditions necessary for continued microbial activity or calcite precipitation.

The data obtained from these tests formed the basis for analyzing variations in CBR values due to bacterial treatment, curing time, compaction energy, and moisture condition. These results were used to evaluate the effectiveness of short-term soil stabilization using *Bacillus tropicus*.

## 2.3 Research Flowchart

Prior to conducting this research, a systematic sequence of research activities was developed to ensure that the study objectives could be achieved efficiently and accurately. The research process began with a preliminary study, which included identification of the research background, problem formulation, and determination of research objectives.

This was followed by a review of relevant literature to support the selection of materials and research methods. Subsequently, soil samples were prepared, treated, and tested in accordance with the planned methodology. The experimental results obtained from laboratory testing were then analyzed and interpreted to evaluate the influence of bacterial treatment and curing time on soil bearing capacity. Finally, conclusions were drawn based on the analysis results.

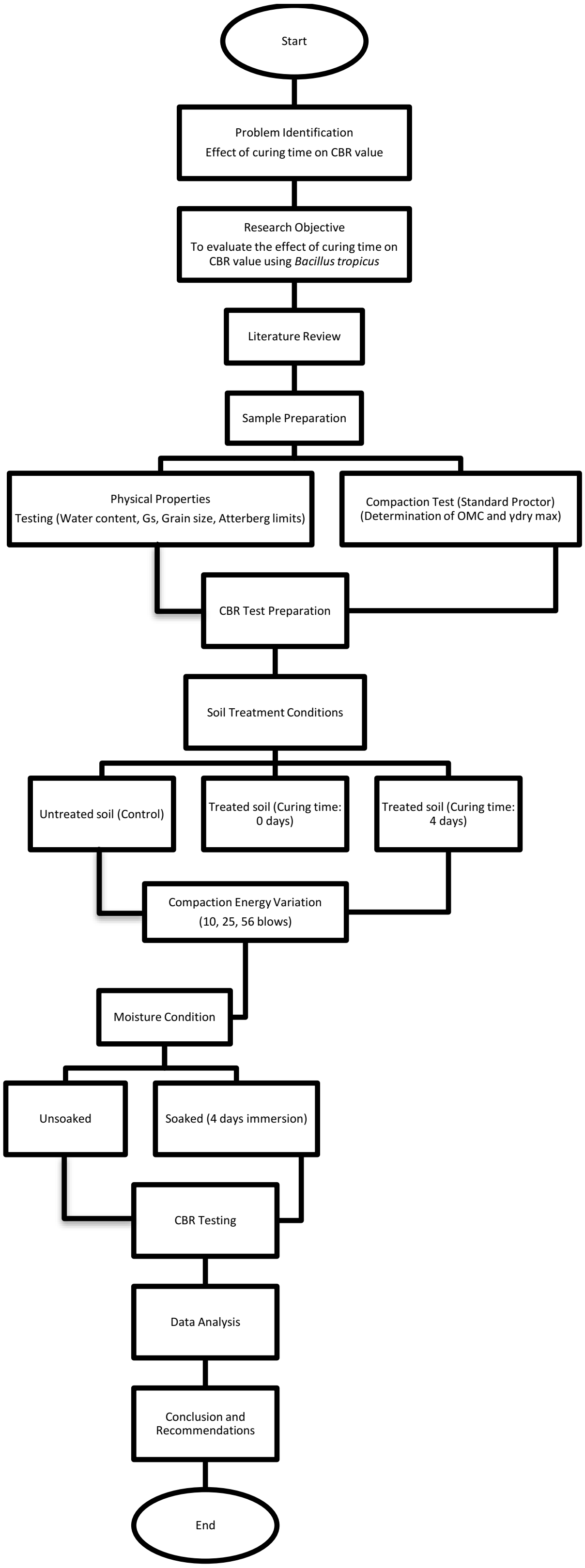


Figure 8. Research Flowchart

## 2.4 Materials

The materials used in this research are as follows:

### 2.4.1 Soil

The soil used in this study was a natural soil collected for laboratory testing. Based on visual observation and preliminary handling, the soil was predominantly sandy but exhibited noticeable cohesion due to the presence of plastic fines. In its natural condition, the soil showed moderate plastic behavior and relatively low bearing capacity, particularly under wet conditions. These characteristics make the soil suitable for evaluating stabilization methods intended to improve subgrade performance.

Detailed soil classification was determined through laboratory testing, including grain size distribution (sieve analysis) and Atterberg limits tests. The test results indicated that the soil is dominated by sand-sized particles with a limited proportion of plastic fines. According to the Unified Soil Classification System (USCS), the soil is classified as **SP–SC (Poorly Graded Sand with Clay)**. Based on the AASHTO classification system, the soil is categorized as **A-2-7**.

This classification indicates a sandy soil whose engineering behavior is significantly influenced by plastic fines. The presence of clayey fines increases moisture sensitivity and leads to a reduction in bearing capacity under soaked conditions. Therefore, the soil is appropriate for investigating stabilization techniques aimed at improving mechanical behavior and bearing capacity when used as subgrade material.

Figure 9. Soil sample

### 2.4.2 *Bacillus tropicus* Bacteria

The biological stabilizing agent used in this study was *Bacillus tropicus*, a bacterium capable of inducing calcium carbonate precipitation through the mechanism of Microbially Induced Calcite Precipitation (MICP). This process enhances soil strength by promoting interparticle bonding and reducing pore spaces within the soil matrix.

The bacteria were obtained from the Biology Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Negeri Makassar. The bacterial cultures were prepared using B4 medium, with the following composition:

1) Urea – 20 g
2) Nutrient Broth – 3 g
3) Sodium Bicarbonate ($NaHCO_3$) – 12 g
4) Calcium Chloride Dihydrate ($CaCl_2 \cdot 2H_2O$) – 14 g
5) Ammonium Chloride ($NH_4Cl$) – 10 g
6) Distilled water (aquades) – 1000 mL

The bacterial solution was incubated for two different curing periods, namely 0 days and 4 days, these curing periods were selected to evaluate the influence of curing time on soil stabilization performance. The prepared bacterial solution was mixed with the soil and used as a stabilizing agent in the treated specimens.


Figure 11. *Bacillus tropicus* culture in B4 medium

## 2.5 Laboratory Equipment

The equipment used in this research is grouped into two categories: for physical testing and mechanical testing of the soil.

*Table 9 .Equipment for Physical Property Testing*

| No. | Type of Test | Equipment |
|---|---|---|
| 1 | *Water "Moisture" Content* |  |
| 2. | *Spesific Gravity* |  |
| *3.* | *Atterberg Limits* |  |

| | | |
|---|---|---|
| 4. | Hydrometer Analysis |  |
| 5. | *Sieve Analysis* |  |

Table 10 .Equipment for Mechanical Property Testing

| No. | Type of Test | Equipment |
|---|---|---|
| 1. | California Bearing Ratio Test |  |
| 2. | *Standar Proctor Test* |  |

## 2.6 Testing Standards

All laboratory testing procedures conducted in this study followed standards issued by the American Society for Testing and Materials (ASTM) and the Indonesian National Standards (SNI).

Table 11 .Standards for Physical Soil Properties Testing

| Type of Test | Test Standards | |
|---|---|---|
| | ASTM | SNI |
| *Water Content* | ASTM D 2216-19 | SNI 1965:2008 |
| *Specific Gravity* | ASTM D 854-14 | SNI 1964:2008 |
| *Sieve Analysis* | ASTM D 6913-17 | SNI 3423:2008 |
| Atterberg Limits | ASTM D 4318 – 10 | SNI 1957:2008 |

Table 12 .Standards for Mechanical Soil Properties Testing

| Type of Test | Test Standards | |
|---|---|---|
| | ASTM | SNI |
| Standard Compaction Test | ASTM D 698-12e2 | SNI 1742:2008 |
| CBR Test | ASTM D 1883 | SNI 1744:2008 |

## 2.7 Soil Characteristics Testing

Before proceeding with mechanical testing, the soil used in this study was subjected to a series of physical property tests to determine its basic characteristics and classification. these tests were conducted to evaluate the soil's index properties and to provide a basis for further analysis related to stabilization performance. the number of test samples used for each laboratory test is presented in Table 13.

Table 13 .Number of Test Samples

| No | Test | Number of Samples |
|---|---|---|
| 1 | Specific Gravity | 3 |
| 2 | Water Content | 3 |
| 3 | Sieve Analysis | 3 |
| 4 | Compaction (Proctor) | 3 |
| 5 | CBR Test (Soaked/Unsoaked) | 6 (Per Condition) |

### 2.7.1 Testing Procedures

The testing process in this study is divided into two main categories:

1. Physical property tests, to classify the soil and understand its fundamental behavior before stabilization.
2. Mechanical property tests, to assess the soil's strength and bearing capacity after curing and also after using *Bacillus tropicus*.

#### 2.7.1.1 Physical Properties Testing

The following tests were conducted to evaluate the fundamental physical behavior of the soil:

1) Water Content Test

The water content test was conducted to determine the amount of water present in the soil, expressed as a percentage of the dry weight of the soil.

Procedure:

a. A representative soil sample was placed in a moisture tin and weighed.
b. The sample was oven-dried at a temperature of 105–110°C for 24 hours.
c. After drying, the sample was weighed again to determine the loss of water.

Water content is an important parameter because it significantly influences soil compaction behavior and strength characteristics.

2) Specific Gravity Test

The specific gravity test was conducted to determine the ratio between the density of soil solids and the density of water.

Procedure:

a. Oven-dried soil passing sieve No. 40 was used for the test.
b. A pycnometer was filled with soil and water, and a series of weight measurements were recorded.
c. Based on these measurements, the specific gravity (Gs) of the soil particles was calculated.

The specific gravity value is required for determining other soil parameters such as void ratio, porosity, and degree of saturation.

3) Sieve Analysis

Sieve analysis was conducted to determine the grain size distribution of the soil.

Procedure:

a. Approximately 500 g of oven-dried soil was placed in a stack of sieves arranged in descending order of sieve size.
b. The sieve stack was mechanically shaken for 10–15 minutes.
c. The mass of soil retained on each sieve was measured to calculate the percentage distribution of particle sizes.

The results of sieve analysis were used to support soil classification based on the USCS and AASHTO systems.

#### 2.7.1.2 Mechanical Properties Testing

Mechanical property tests were conducted to evaluate the engineering behavior of the soil, particularly its strength and load-bearing capacity. These tests were performed on untreated soil as well as soil treated with *Bacillus tropicus* to assess the effectiveness of biological stabilization.

1) Standard Proctor Compaction Test

The Standard Proctor Compaction Test was conducted to determine the optimum moisture content (OMC) and maximum dry density (MDD) of the soil.

Procedure:

a. Soil samples were compacted in a standard mold in three equal layers.
b. Each layer was compacted using a standard hammer dropped from a fixed height, following ASTM D698.

c. Several trials were conducted at different water contents to establish the relationship between moisture content and dry density.

The results of this test are essential because soil compacted near its OMC exhibits higher strength, improved bearing capacity, and reduced compressibility.

2) California Bearing Ratio (CBR) Test *(Main focus of this study)*

The California Bearing Ratio (CBR) test was conducted to evaluate the load-bearing capacity of the soil by measuring its resistance to penetration relative to a standard crushed stone material. This test served as the primary method for assessing soil strength improvement in this research.

Procedure:

1. Soil samples were compacted in CBR molds using three compaction energy levels: 10, 25, and 56 blows per layer.
2. For treated samples, two curing conditions were applied: 0 days curing (specimens were tested immediately after compaction, with no curing period) and 4 days curing (specimens were left at room temperature for four days prior to testing).
3. CBR tests were conducted under both soaked and unsoaked conditions.
4. A standard penetration piston was driven into the specimen at a constant rate, and corresponding load values were recorded.

The following sample categories were tested:

- Untreated soil (control sample)
- Soil treated with *Bacillus tropicus* – curing time 0 days
- Soil treated with *Bacillus tropicus* – curing time 4 days

## *2.8* Bacteria Culture Process

The *Bacillus tropicus* bacteria used in this study were prepared through a controlled culture process prior to application. The bacterial culturing was conducted using B4 nutrient medium to promote bacterial growth and activity.

The composition of the B4 medium was as follows:

- Urea – 20 g
- Nutrient Broth – 3 g
- $NaHCO_3$ – 12 g
- $CaCl_2 \cdot 2H_2O$ – 14 g
- $NH_4Cl$ – 10 g
- Distilled water – 1 liter

All ingredients were mixed thoroughly in a sterilized glass container to ensure homogeneity. The solution was then placed in a shaker incubator and maintained under controlled conditions for a period of 4 days to allow bacterial growth and multiplication. For the 0-day curing group, the bacterial solution was prepared and applied to the soil on the same day, without any pre-incubation of the compacted specimen; specimens were compacted and immediately subjected to testing or soaking. For the 4-day curing group, compacted specimens were allowed to rest at room temperature for four days prior to testing or soaking.

The resulting bacterial culture solution was used directly as a biological stabilizing agent in the soil treatment process.

## 2.9 California Bearing Ratio CBR Sample Preparation

The preparation of soil specimens for the California Bearing Ratio (CBR) test was conducted in several systematic stages to ensure consistency and repeatability. Three different soil treatment conditions were prepared:

1. Untreated soil (control group)
2. Soil treated with *Bacillus tropicus* – curing time 0 days
3. Soil treated with *Bacillus tropicus* – curing time 4 days

Each treatment group was tested under both soaked and unsoaked conditions and compacted using Standard Proctor compaction energies: 10, 25, and 56 blows per layer.

- Maximum Dry Density = $1.275\ g/cm^3$
- Optimum Moisture Content = 27 %
- CBR Mold Diameter = $15.5\ cm$
- CBR Mold Height = $17.5\ cm$

1.1 Mold volume calculation:

The volume of the CBR mold was calculated using the standard formula for the volume of a cylinder

$$V = \frac{1}{4}\pi d^2 h = \frac{1}{4} \times \pi \times 15.5^2 \times 17.5 = 3300.43\ cm^3$$

Where:
$d$ = diameter of mold $(cm)$
$h$ = height of mold $(cm)$

The diameter of the mold was $15.5\ cm$ , and the height was $17.5\ cm$ , When these dimensions were inserted into the formula, the total volume was found to be approximately $3300.43\ cm^3$.

2.1 Dry soil weight calculation

To determine how much dry soil was needed, the volume of the mold was multiplied by the maximum dry density of the soil. The dry density used was 1.275 g/cm³, which gave a dry soil weight of around 4208.05 grams

$$W_s = \gamma_{dry\ max} \times V$$

$$W_s = 1.275 \times 3300.43$$

$$W_s = 4208.05\ g$$

3.1 Calculating Water Weight

Water was added based on the optimum moisture content (OMC), which was 27%. However, since 6% of the weight would be replaced by bacteria solution, the water needed was adjusted to only 21% of the dry soil weight. This gave approximately 883.691 grams of water.

$$W_W = (\omega_{opt} - \text{Bacteria content}) \times W_S$$
$$W_w = 21\% \times 4208.05$$
$$W_w = 883.69\ g$$

4.1 Bacteria solution

$$W_{bacteria} = 6\% \times W_s$$
$$W_{bacteria} = 6\% \times 4208.05$$
$$W_{bacteria} = 252.48\ g$$

5.1 Mixing Process:

The dry soil was first mixed with the calculated amount of water until a uniform moisture distribution was achieved. Subsequently, the bacterial solution was added, and the mixture was thoroughly blended until a homogeneous consistency was obtained.

6.1 Compaction:

The soil mixture was compacted inside the mold in three layers, with each layer being compacted using a Proctor hammer. Three different compaction energy levels were applied: 10 blows, 25 blows, and 56 blows per layer.

7.1 Curing:

After compaction, the samples were left to cure at room temperature for 4 days. During this period, dial gauge readings were taken every day to monitor changes.

8.1 Soaking:

For samples to be tested under soaked conditions, they were submerged in water for 96 hours (4 days) before testing to simulate saturated field conditions.

9.1 CBR Testing and the procedure:

The CBR test was conducted in accordance with applicable ASTM and SNI standards. The test involved placing a penetration plunger at the center of the compacted specimen and applying a vertical load at a constant penetration rate of 1.25 mm/min.

Load readings were recorded at standard penetration depths of 2.5 mm and 5.0 mm, and the CBR value was calculated by comparing the measured load to the corresponding standard load.