

Bio-Enzymes: A Sustainable Solution for Strengthening Black Cotton Soil

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Abstract:-

This manuscript presents a comprehensive study which explores the potential of bio enzymes, derived from organic waste materials, as an alternative, sustainable solution for soil stabilization. Soil stabilization is a critical process in civil engineering, particularly for improving the strength and stability of soil used in construction projects. Conventional methods of stabilization, including the use of cement, lime, and bitumen, have raised environmental and economic concerns due to their high carbon footprint, non-biodegradability, and the depletion of natural resources. The primary aim of this study, "**Bio enzymes: A Sustainable Solution for Strengthening Black Cotton Soil**" is to investigate the effectiveness of bio enzymes in enhancing the geotechnical properties of various soil types. Bio enzymes are natural catalysts that promote biochemical reactions in the soil, improving its compaction, strength, and permeability. This project focuses on developing bio enzymes through a fermentation process using organic waste like citrus peels, sugarcane molasses, and other agricultural by-products.

Key words: Bio-enzymes, Black cotton soil, Soil stabilization, Sustainable construction, Waste utilization.

1. Introduction

The development of sustainable infrastructure is a pressing need in today's world, driven by challenges such as population growth, urbanization, and environmental concerns. Soil stabilization plays a critical role in civil engineering, as the strength and durability of soil directly influence the stability of structures built upon it. Traditional stabilization methods often involve the use of cement, lime, or other chemical additives, which, while effective, pose significant environmental challenges due to high carbon emissions and soil toxicity. This necessitates the exploration of innovative and sustainable alternatives.

Need of soil stabilization using bio-enzyme

Soil stabilization is a crucial aspect of civil engineering, particularly in geotechnical and foundation engineering, where the strength, durability, and stability of soil play a critical role in the overall safety and performance of infrastructure projects. The traditional methods of soil stabilization, such as the use of lime, cement, or bitumen, have long been the go-to solutions. However, these methods have certain limitations, including high costs, adverse environmental impacts, and sustainability concerns.

Consequently, there is a growing need to explore alternative, eco-friendly solutions for soil stabilization that not only address engineering requirements but also contribute to sustainable development goals.

The primary objective of soil stabilization is to enhance the engineering properties of soil, such as its strength, compaction, permeability, and load-bearing capacity, to make it suitable for construction activities. Expansive soils, for instance, which are prone to swelling and shrinkage due to moisture variation, present a significant challenge in many areas. These soils can lead to cracks in buildings, roads, and pavements, resulting in costly repairs and compromising the safety of structures. In such cases, effective stabilization techniques are essential to prevent these issues.

Bioenzymes are natural catalysts derived from organic waste materials, such as fruit waste, agricultural by-products, or sugarcane molasses. When applied to soil, bioenzymes enhance its physical and chemical properties by promoting biochemical reactions that strengthen soil particles, reduce permeability, and improve compaction. Research has shown that bioenzymes can improve various geotechnical properties of soil, California Bearing Ratio (CBR), and swelling potential. For example, studies by Agarwal and Kaur (2021) and Taha et al. (2021) have demonstrated that enzyme-treated soils exhibit enhanced mechanical properties, making them more suitable for construction applications. Moreover, the use of bioenzymes reduces the reliance on non-renewable resources, contributing to a more sustainable approach to infrastructure development. The production of bioenzymes from organic waste materials is relatively low-cost, and the application process is simpler compared to that of traditional chemical stabilizers. This makes bioenzyme-based stabilization an attractive option, particularly for developing countries and regions with limited access to expensive stabilization materials.

Problem statement

The Peth – Sangli State Highway has persistent problems of road stabilization due to black cotton soil being the base strata. Moreover, this particular area is renowned geologically for black cotton bed up to considerable depth. These traditional stabilization methods do not work or are inappropriate for this soil type which results in road swelling, shrinkage, reduced load bearing capacity, compromising road safety, longevity and strength. Therefore, a solution must focus on developing emerging methodologies that are renewable, ecologically benign, and radical. This project intends to stabilize black cotton soil using spent wash and citrus waste by creating bio-enzymes, thus these organic components will enhance the soil's bearing capacity increasing the road's durability.

Scope of project

This scope encompasses formulating and evaluating these bio-enzymes with the aim of enhancing soil's physical characteristics succumbing to problems like swelling and shrinkage which ultimately endanger road construction's feasibility. Activities include lab analysis, preliminary field tests, and continuous evaluation to determine how effective this approach is towards sustainability and resiliency. Furthermore, the project seeks to determine the ecological consequences, soil waste management economy, and level of control organic waste using for soil consolidation possesses in starting for soil waste management policies and objectives.

Materials and Proportions



Fig.01: Proportion of Materials

01. Spentwash (Molasses):

Molasses, a viscous byproduct from sugarcane processing, is a cornerstone material in this project due to its high carbohydrate content and nutritional value for microbial activity. It serves as a rich source of fermentable sugars—mainly sucrose, glucose, and fructose—which are crucial for supporting the growth and multiplication of microorganisms during bioenzyme production. The presence of trace minerals and organic compounds further supports enzymatic reactions, ensuring efficient fermentation. Its high viscosity helps regulate the fermentation process, allowing for controlled and consistent enzyme synthesis over time. In addition to its functional role, the use of molasses aligns with the project's sustainability objectives, as it transforms an industrial byproduct into a valuable input, reducing waste in the sugar industry. Economically, molasses is cost-effective and readily available, making it an ideal choice for scaling bioenzyme production for large-scale applications. Its use not only minimizes waste but also supports the circular economy, turning byproducts into functional resources for sustainable construction practices.

02. Citric peels:

Citrus waste, comprising peels, pulp, and other byproducts of citrus fruit processing, plays a pivotal role in enhancing the fermentation process. The natural acidity of citrus waste creates a favorable environment for microbial activity, lowering the pH to optimal levels for enzyme production. Its organic composition, rich in vitamins, minerals, and bioactive compounds, contributes additional nutrients that stimulate microbial growth, leading to higher enzyme yields. Moreover, citrus waste contains natural oils and flavonoids, which may have auxiliary benefits in promoting enzyme stability during production. By incorporating citrus waste, this project leverages an abundant organic material that would otherwise contribute to landfill waste, aligning with principles of sustainable waste management. Its biodegradability ensures that no harmful residues are left behind, making it a green alternative in bioenzyme production. The use of citrus waste also adds diversity to the nutrient profile of the fermentation mixture, complementing the sugars provided by molasses and ensuring a balanced medium for microorganisms to thrive.

03. Water:

Water serves as a critical enabler throughout the entire process, acting as a medium for fermentation and an agent for bioenzyme application. During the production of bioenzymes, water dissolves molasses and citrus waste, creating a uniform mixture that promotes efficient microbial activity. It also ensures optimal pH levels and temperature regulation within the fermentation setup, both of which are essential for maintaining a robust microbial ecosystem. The quality of water used is paramount—free of contaminants or impurities—to prevent interference in the fermentation process, which could compromise the yield and quality of the bioenzymes. In the application phase, water is instrumental in diluting the concentrated bioenzyme solution to the desired consistency, enabling uniform application across the soil. This ensures thorough penetration into the soil structure, allowing bioenzymes to interact with particles effectively. Additionally, water facilitates the transportation of enzymes within the soil, aiding in the stabilization process. The dual role of water in production and application underscores its indispensability in this project.

Methodology

01. Collection of soil sample and citrus waste:

To evaluate the effectiveness of bio-enzyme in stabilizing soil, samples were collected from a nearby agricultural field. Also, citrus peels (mostly orange and lemon) were collected at no charge from local juice centers. These were supplemented with sugarcane molasses from Rajarambapu sugarcane factory, Islampur. These organic waste materials were chosen because of their accessibility and high enzyme-producing potential, thus complementing the aim of the project in terms of sustainability and low cost.

02. Tests conduction on untreated soil:

Before applying the bio-enzyme treatment, the natural engineering properties of the soil were evaluated. Soil samples were first sun-dried and then tested using a range of geotechnical methods in line with established standards. The following tests were carried out on the untreated soil to gain a baseline understanding of its behavior:

- **Sieve Analysis** – Conducted to determine the soil's particle size distribution.
- **California Bearing Ratio (CBR) Test** – Used to evaluate the soil's load-bearing capacity.
- **Liquid Limit Test** – Performed to identify the moisture content at which the soil transitions from a plastic to a liquid state.
- **Plastic Limit Test** – Assessed to determine the water content at which the soil shifts from a semi-solid to a plastic consistency.
- **Permeability Test** – Measured the soil's ability to allow water to pass through, indicating its drainage potential.

03. Preparation of bio-enzyme sample:

The bio-enzyme solution was synthesized from sugarcane molasses, citrus waste- specifically peels of lemons and oranges, and water according to the volume ratio of 1:3:10. Yeast was added to the blend to speed up the fermentation process. The mixture was stored in a sealed container and allowed to ferment at room temperature for 21 days. Throughout this period, the mixture was stirred from time to time to promote microbial activity as well as uniform fermentation in the entire solution. This solution served as an eco-friendly stabilizing agent for treatment and analysis of soil samples.

04. Mixing of various proportions of bio-enzyme sample and water with soil:

After the fermentation period, the prepared bio-enzyme solution was mixed with water and applied to the untreated soil in varying proportions to study the impact of different concentrations on soil stabilization. The enzyme-water mixtures were thoroughly blended with soil samples and stored under controlled conditions. The treated soil samples were cured for varying durations to assess how the length of curing influenced the enhancement of soil properties. This approach helped analyze the optimum bio-enzyme dosage and curing period for maximum stabilization effectiveness.

05. Tests conduction on treated soil:

To evaluate the effectiveness of the bio-enzyme treatment, the same series of geotechnical tests conducted on the untreated soil were repeated on the treated soil samples. The tests were carried out after each curing period to monitor improvements in soil properties over time. A comparative analysis of untreated and treated samples offered valuable insights into how bio-enzyme application affected soil strength, consistency, and permeability.

06. Determination of most effective proportion:

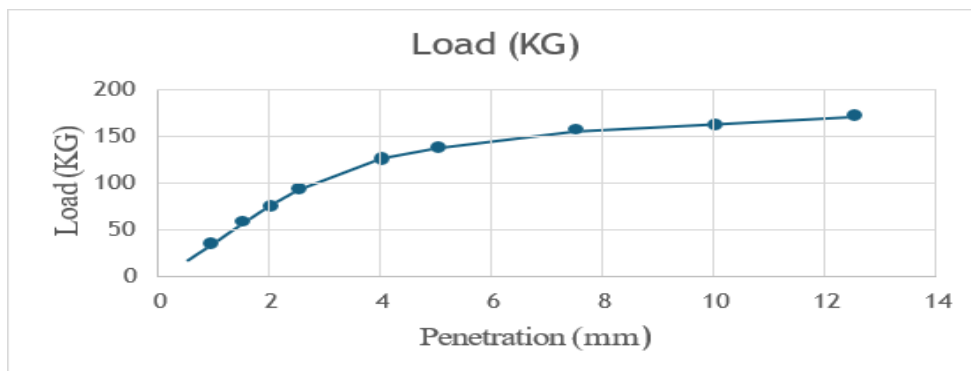
All test results obtained from the treated soil samples were carefully analyzed and compared with those of the untreated soil. The performance of each treatment combination based on varying bio-enzyme proportions and curing durations was evaluated to determine the most effective mix. The treatment that showed the greatest improvement in soil strength, plasticity characteristics, and permeability was identified as the optimal solution for soil stabilization using bio-enzymes.

Tests on untreated soil**01. Sieve analysis test**

Sr no.	IS sieve	Mass Retained (gm)	% Retained (g)	Cumulative % Retained (gm)	% finer
1	4.75 mm	0.084	8.4	8.4	91.6
2	2.36 mm	0.066	6.6	15	85
3	1.18 mm	0.158	15.8	30.8	69.2
4	600	0.044	4.4	35.2	64.8
5	300	0.190	19	54.2	45.8
6	150	0.368	36.8	91	9
7	75	0.028	2.8	93.8	6.2
8	pan	0.059	5.9	99.7	0.3

Conclusion: Cu & Co is between 1 and 3, given soil sample is well graded soil with little fines.

02. California bearing ratio (CBR) test:



Sr. No.	Penetration (mm)	Load (KG)
1	0.5	16
2	1.0	35.5
3	1.5	56
4	2.0	75.5
5	2.5	92
6	4.0	126
7	5.0	137
8	7.5	155
9	10	162.5
10	12.5	170.5

Conclusion: For pavement construction, minimum soil bearing capacity should be 8%. But, SBC of sample is 9.53 %. So, no stabilization is needed in this case.

03. Permeability (Falling Head Method):

Sr no.	Initial Head (H1)	Final Head (H2)	Elapsed Time	Log (H1/H2)	Coefficient (k)
1	100	95	10	0.022	2.578×10^{-3}
2	95	91.2	10	0.017	1.992×10^{-3}
3	91.2	87.3	10	0.018	2.110×10^{-3}

Table 01: Permeability results for D= 2cm

Sr no.	Initial Head (H1)	Final Head (H2)	Elapsed Time	Log (H1/H2)	Coefficient (k)
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1	100	63	10	0.200	1.463×10^{-3}
2	63	38.1	10	0.218	1.595×10^{-3}
3	38.1	19.7	10	0.286	2.092×10^{-3}

Table 02: Permeability results for D= 0.5cm

Conclusion: Coefficient of permeability shows soil sample used is clayey soil and has low permeability.

04. Plastic limit:

CONTAINER NO	1	2	3
Mass of empty container (M1)	8	16	14
Mass of empty container & wet soil (M2)	18	22	22
Mass of empty container & dry soil (M3)	15	19.35	21
Mass of dry soil	7	3.35	7
Mass of water	3	2.64	1
Water content	0.45	0.78	0.14

Table 03: Plastic limit test calculations

Average = $(0.45+0.78+0.14)/3 = 0.28=28\%$

Plasticity index (Ip)= WL-Wp = $0.45-0.28 = 0.17= 17\%$

Conclusion: As plasticity index is 17% then given soil sample is medium plastic and type of soil is silty clay.

05. Liquid limit:

DETERMINATION NO	1	2	3
No of blows	40	28	29
Container no	1	2	3
Mass of empty container	22	24	21
Mass of empty container + wet soil	40.7	42.14	38.10
Mass of empty container + dry soil	36.32	36.43	32.46
Mass of dry soil	13.37	12.43	11.46
Mass of water	5.98	5.71	5.64
Water content	40.39	45.93	49.21

Table 04: Liquid limit test calculations

Average = $(40.39+45.93+49.21) \div 3 = 45.17\%$

Conclusion: Liquid limit of given sample is 45.17% and has intermediate plasticity.

Untreated soil properties

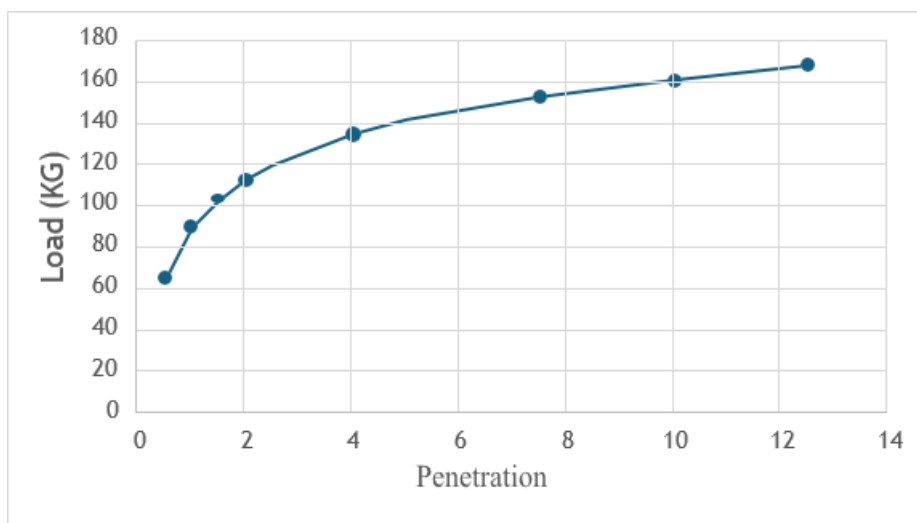
Parameter	Value	Soil Classification	Engineering Interpretation
CBR (%)	9.53	Clayey soil	Moderate strength; improvement needed
Permeability (cm/s)	1.97×10^{-3}	Low permeability (clayey)	Poor drainage; water retention risk
Plastic Limit (%)	28	Medium plasticity	Deformable; moisture-sensitive
Liquid Limit (%)	45.17	Intermediate plasticity	Unstable at high water content

Table 05: Untreated soil properties

Tests on treated soil:

01. California Bearing Ratio (CBR):

Sr. No.	Penetration (mm)	Load (KG)
1	0.5	64
2	1.0	89
3	1.5	102
4	2.0	112
5	2.5	119
6	4.0	134
7	5.0	141
8	7.5	152
9	10	160
10	12.5	167



Conclusion: SBC of sample is 12.33 %. The treated soil exhibited a high bearing capacity, indicating its suitability for use in subgrade layers of pavements.

02. Permeability (Falling Head Method):

For D= 2cm.

Sr. No	Initial Head	Final Head	Elapse Time	Log(H1/H2)	Coeff. of Permeability
1	100	96	10	0.018	3.12×10^{-4}
2	96	92.2	10	0.017	3.00×10^{-4}
3	92.2	88.6	10	0.017	3.04×10^{-4}

Table 06: Permeability results for D= 2cm

For D= 0.5cm.

Sr. No	Initial Head	Final Head	Elapse Time	Log(H1/H2)	Coeff. of Permeability
1	100	74	10	0.130	2.98×10^{-4}
2	74	55	10	0.130	3.05×10^{-4}
3	55	40	10	0.140	3.10×10^{-4}

Table 07: Permeability results for D= 0.5cm

Average values:

$$K (D= 2\text{cm})= 3.05 \times 10^{-4} \text{ cm/sec.}$$

$$K (D= 0.5\text{cm})= 3.04 \times 10^{-4} \text{ cm/sec.}$$

$$\text{Average } K= 3.05 \times 10^{-4} \text{ cm/sec.}$$

03. Plastic Limit (PL):

Container no	1	2	3
Mass of empty container with lid (M1)	8.0	16.0	14.0
Mass of container with lid + wet soil (M2)	18.2	22.5	22.1
Mass of container with lid + dry soil (M3)	15.3	19.7	20.0
Mass of dry soil (Md)= M3-M1	7.3	3.7	6.0
Mass of water (Mw)= M2-M3	2.9	2.8	2.1
Water content (W)= (M2-M3) / (M3-M1)	23.29	21.62	22.29

Table 08: Plastic limit test calculations

$$\text{Average} = (23.29 + 21.62 + 22.29) / 3 = 22.4\%$$

04. Liquid Limit (LL):

Determination no	1	2	3
No of blows	38	27	29
Container no	1	2	3
Mass of empty container with lid (M1)	22	24	21
Mass of container with lid + wet soil (M2)	40.20	42	38.30
Mass of container with lid + dry soil (M3)	36.40	36.80	32.70
Mass of dry soil (Md)= M3-M1	14.40	12.28	11.70
Mass of water (Mw)= M2-M3	3.80	5.20	5.60

Water content (W)	39.80	40.60	41.55
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Table 09: Liquid limit test calculations

Average= (39.80+40.60+41.55) / 3= 40.65%

Treated soil properties

Parameter	Value	Soil Classification	Engineering Interpretation
CBR (%)	12.33 %	High strength, improved bearing capacity	Suitable for subgrade layers in roads and pavements
Permeability (cm/s)	3.05×10^{-4}	Low permeability, dense clayey structure	Better resistance to water infiltration and improved stability
Plastic Limit (%)	22.4	Low plasticity, less moisture sensitivity	Reduced deformation under stress and better workability
Liquid Limit (%)	40.65	Intermediate plasticity, Stable behavior	Lower water retention; enhanced structural integrity in wet conditions

Table 10: Treated soil properties

Calculating for 1km stretch of road:

- Length = 1 km = 1000 meters
- Width = typical single-lane rural road \approx 3 meters
- Depth of treatment = approx. 0.2 meters (20 cm)

Volume = Length \times Width \times Depth

= 1000 m \times 3 m \times 0.2 m

= **600 cu. m**

So,

3 liters (0.003 cu.m) solution \rightarrow 0.01 cu.m soil So per **1 cu.m** soil:

= (0.003 / 0.01) \times 1 cu.m = **0.3 cu.m** solution (i.e., 300 liters) Now,

= 600 \times 300 liters = **180,000 liters** of enzyme solution

Ratio is:

30 ml enzyme : 2970 ml water

→ 1% enzyme concentration

So for 180,000 liters (i.e., **180,000,000 ml**) solution:

- Enzyme = 1% of 180,000,000 ml = **1,800,000 ml = 1800 liters**
- Water = 180,000 – 1800 = **178,200 liters**

So,

To treat **1 km soil road** (assuming 3 m width and 20 cm depth), you'll need approximately.

- 1800 liters of enzyme
- 178,200 liters of water
- Total solution: **180,000 liters**

Cost estimation:

Ingredient	Quantity per L	Approx Cost	Total
Molasses	~250 ml	₹15/liter	₹3.75
Citrus waste	~250 g	Free (waste)	₹0
Yeast (optional)	~2 g	₹500/kg	₹1.00
Container + Storage	-	-	₹1.00

Table 11: Cost estimation

Approx. ₹6 per liter

So, 1800 L × ₹6 = ₹10,800

If water is available freely (e.g., borewell or recycled) then cost = Negligible, If purchased: say ₹10 per 1000 liters = ₹1 per 100

178,200 L ≈ ₹1,800 (if purchased)

We'll take a conservative estimate: ₹1,500

- Labor & Machinery Cost
- 1 tractor or water tanker to spray + mix
- 1 soil compactor (vibratory roller)
- 1 driver/operator each
- Total working time = 2 days Estimated cost:
- Vehicle + driver (2 days) = ₹4,000
- Compactor + operator (2 days) = ₹6,000
- Manual labor (4 persons × ₹500 × 2 days) = ₹4,000

Total = ₹14,000

- Miscellaneous (transportation, storage, losses) Let's keep 10% buffer = ₹2,500
- Final Estimated Cost for 1 km Soil Road Treatment

Component	Cost (INR)
Enzyme Production	₹10,800
Water (if applicable)	₹1,500
Labor + Equipment	₹14,000
Miscellaneous	₹2,500
Total	₹28,800

Table 12: Final Cost**Conclusion:****01. Sustainable Ground Improvement:**

This project establishes the potential of using a bio enzyme derived from fermented sugarcane molasses and citrus waste as a sustainable and eco-friendly solution for soil stabilization, particularly suited for rural development.

02. Effective for Village Roads on Black Cotton Soil:

The enzyme treatment increased the strength and performance of black cotton soil, which is one of the most difficult soil types due to its expansive characteristics, making it more reliable for utilization as construction material for low-cost village roads.

03. Enhanced Moisture Resistance:

The treated soil displayed reduced permeability, improving resistance to water infiltration — a key factor in ensuring long-term durability of unpaved rural roads under variable weather conditions.

04. Improved Soil Plasticity:

The reduction in plastic and liquid limits indicates better workability and reduced shrink-swell behavior, which is crucial for minimizing cracking and deformation in village roads built on expansive soils.

05. Utilization of Local Organic Waste:

The bioenzyme was produced from easily available organic waste, promoting a waste-to-resource model that supports local sustainability, low cost, and reduced environmental impact.

06. Simple, Scalable, and Community-Oriented:

The method is low-tech, affordable, and easily adaptable in rural regions, making it a practical and scalable solution for enhancing road infrastructure in villages while maintaining ecological balance.

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