

Bio-Enzymatic Treatment of Expansive Black Cotton Soil Using TerraZyme and Fly Ash: Quantifying the Strength–Swelling Trade-off

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ABSTRACT: Black Cotton Soil (BCS), prevalent across central India, poses severe geotechnical challenges owing to its high plasticity, low bearing capacity, and pronounced swelling behaviour. Fly ash and bio-enzyme stabilizers have each been shown to improve expansive soils, but the two have not previously been optimised sequentially on the same highly expansive soil, and the interaction between them has not been quantified. This study reports a three-phase laboratory programme on a CH black cotton soil (LL = 61%, PI = 38%, FSI = 66.67%). Phase 1 optimised the TerraZyme (TZ) dosage on untreated BCS; Phase 2 added Class-F fly ash (FA) at 10–40% by dry weight to the TZ-optimised soil; Phase 3 measured swelling magnitude and swelling pressure on the three key mixes. The replicate scheme is stated in full in Section 3.6: one specimen per mix per condition for UCS and CBR, and two for the swelling tests. Phase 1 identified 67 ml/m³ as the optimum TerraZyme dosage, raising Day-0 UCS from 117.44 to 838.24 kN/m² (7.1×), Day-7 UCS from 134.38 to 1205.43 kN/m² (9.0×), soaked CBR from 3.28% to 13.87% (4.2×) and unsoaked CBR from 13.42% to 16.24% (+21%), while reducing the plasticity index from 38% to 23%. Phase 2 identified 20% Class-F fly ash as the best-performing addition on strength and plasticity criteria, and Phase 3 confirmed that this mix reduced swelling pressure from 2.40 to 1.35 kg/cm² (–43.8%) and swelling magnitude by 50%. However, the fly ash addition was strongly antagonistic to bearing capacity: relative to the TerraZyme-only optimum the Day-7 UCS fell by 70%, and both the soaked (1.82–2.55%) and the unsoaked (6.37–9.12%) CBR of every TZ + FA mix fell below the corresponding values of the untreated soil. The combined mix is therefore not recommended as a load-bearing pavement subgrade. TerraZyme alone at 67 ml/m³ is the appropriate treatment where bearing capacity governs, whereas the TZ + 20% FA combination is suited only to applications in which volume stability governs and the treated layer is not required to carry traffic loading, such as expansive-soil cover or cushion layers, canal linings and non-structural fills. Quantifying and mechanistically explaining this strength–swelling antagonism, rather than recommending the combined mix, is the principal contribution of this study.

KEYWORDS: Black Cotton Soil, TerraZyme, Fly Ash, Swelling Pressure, CBR, UCS, Bio-Enzyme Stabilization, Antagonistic Interaction, and Subgrade Design.

1. INTRODUCTION

1.1 Expansive soils and the black cotton soils of central India

A frequently costly geotechnical issue in civil engineering is expansive soils, which swell in the presence of moisture and contract in its absence, leading to differential settlements, pavement cracking, and foundation distress. The annual global economic impact of expansive soils exceeds the combined losses from floods and earthquakes (Jones and Holtz, 1973; Gromko, 1974). Black cotton soil (BCS), a product of Deccan basalt, is spread over approximately 51.8 million hectares in India across Maharashtra, Madhya Pradesh, Karnataka, Gujarat, Andhra Pradesh, and parts of Uttar Pradesh (Ranjan and Rao, 2016). It has a high liquid limit (50–80%), high plasticity index, low bearing capacity, and high free swell index (greater than 50), classifying it as expansive per IS 2720 (Part 40). Infrastructure constructed on BCS — especially road pavements, canal linings, and light-load structures — experiences frequent seasonal distress.

1.2 Conventional stabilization and its limitations

Traditional stabilizers (mainly cement and lime) have been used to reduce plasticity and enhance compaction, UCS, and CBR. The cementitious reactions (C–S–H and C–A–H gels) responsible for densification of cement-treated soils were reported by MacLaren

and White (2003), whereas Nelson and Miller (1992) described how lime-induced cation exchange and flocculation reduce plasticity before long-term pozzolanic hardening occurs. Nevertheless, these techniques have serious disadvantages: high energy consumption, CO₂ emission, susceptibility to sulfate-induced ettringite heave, high cost that limits applicability in rural settings, and potential groundwater contamination from chemical leachate (Verma et al., 2021; Utkarsh and Jain, 2025).

1.3 Bio-enzymatic stabilization

These constraints have driven the search for environmentally friendly alternatives such as bio-enzymatic stabilization. Bio-enzymes are organic catalysts that reduce the cation-exchange capacity of clay minerals by coating particle surfaces, thereby compressing the electrical double layer and reducing water affinity — resulting in lower plasticity, improved compaction, and increased strength (Gupta et al., 2017; Sethi et al., 2021). TerraZyme is a fermented extract of vegetables and fruits used for stabilization of pavement subgrades (TerraZyme Safety Data Sheet, Nature Plus; Bergmann, 2000); it is accredited by the Indian Roads Congress. Shukla et al. (2003) established that bio-enzyme road construction is significantly cheaper than lime-stabilized construction. Dhinakaran and Prasanna (2007) used laboratory and field observations to establish the relationship between enzymatic treatment, reduced

OMC, and enhanced compaction. Gupta et al. (2017) developed dosage–response relations for TerraZyme-treated soils. Muguda and Nagaraj (2019) showed that enzymatic treatment of an earthen construction material lowered the plasticity index and increased strength, and emphasised that the magnitude of the improvement is governed by the clay mineralogy of the host soil rather than by the enzyme alone. Panchal et al. (2017) determined that progressive strength improvements occurred with curing, with diminishing returns beyond 400 ml/m³. Agarwal and Kaur (2014) found progressive improvement of UCS in BCS using TerraZyme. Rajoria and Kaur (2014) established a lowering of the Atterberg limits and improvement in CBR for various soils. Banakar and Nandini (2018) showed enhanced compaction and strength using TerraZyme and hypo-sludge. Singh (2020) demonstrated that enzymatic treatment is complementary to low-carbon cementitious by-products such as cement kiln dust. Kolhe and Dhatrak (2022) confirmed that 7-day curing gives significantly higher UCS compared to immediate testing. Aswathy et al. (2021) found that kaolinite clay achieved the best UCS when using TerraZyme–lime mixtures, with an optimum enzyme dosage of 0.1 ml/kg.

1.4 Fly ash stabilization of expansive soils

Class-F fly ash, a pozzolanic by-product of coal combustion produced in India at over 200 million tonnes annually (Haque, 2013), is also well-established as an expansive soil stabilizer. Phanikumar and Sharma (2007) documented reduced volume-change behaviour and enhanced strength in fly ash-treated expansive clays, reporting that both swell potential and swelling pressure decreased by approximately 50% at 20% fly ash content when compared at constant dry unit weight, but increased by approximately 60% when compared at constant weight of clay — a distinction that is central to the interpretation of the present results. Phani Kumar and Sharma (2004) explained pozzolanic binding and dilution of active clay as the mechanisms behind plasticity and swelling reduction. Noaman et al. (2022) conducted an exhaustive review of fly ash efficiency on high-plasticity clays. Das and Maharana (2019) studied TerraZyme–fly ash–soil mixes for road construction, while Raghwa and Sonthwal (2021) noted the feasibility of combined fly ash–TerraZyme stabilization of highway subgrades.

1.5 Research gap, novelty and objectives

The bio-enzyme literature and the fly ash literature have developed largely in parallel, and the small number of studies that combine the two (Das and Maharana, 2019; Aswathy et al., 2021; Raghwa and Sonthwal, 2021) share three specific shortcomings. First, the enzyme dosage is adopted from the manufacturer's recommendation or from an unrelated soil rather than being optimised on the host soil, so the fly ash effect and the enzyme effect cannot be separated. Second, volume-change behaviour is reported through the free swell index only; swelling pressure measured under the constant-volume oedometer condition of IS 2720 (Part 41) — the quantity actually required for subgrade and cover-layer design — is almost never reported for enzyme–fly ash systems. Third, and most consequentially, soaked CBR is frequently omitted, so any adverse effect of the fly ash on the saturated bearing capacity remains invisible.

The present study was designed to close these three gaps simultaneously on a single, well-characterised, highly expansive soil (CH, LL = 61%, PI = 38%, FSI = 66.67%). Its specific contributions are: (i) the TerraZyme dosage is optimised independently on the untreated BCS and then held fixed, so that every subsequent change can be attributed unambiguously to the fly ash; (ii) plasticity, compaction, UCS at two curing ages, soaked and unsoaked CBR, swelling magnitude and swelling pressure are measured on the same set of mixes, allowing the complete strength–bearing–volume-change trade-off to be quantified on one soil; (iii) a normalised performance framework (Figure 7) and a moisture-sensitivity index (the ratio of soaked to unsoaked CBR, Table 3 and Figure 5b) is introduced so that the competing effects can be compared on a

common basis; (iv) an explicit dosing protocol is given that converts the volumetric enzyme dosage (ml/m³) into the quantity added per specimen, which the existing literature does not report and without which the results are not reproducible; and (v) an antagonistic interaction between TerraZyme and Class-F fly ash is documented and mechanistically explained — the fly ash suppresses swelling but drives both soaked and unsoaked CBR below the values of the untreated soil. This negative finding, and the design guidance that follows from it, is the principal contribution of the paper.

The investigation was carried out on BCS from the MANIT Bhopal campus and Class-F fly ash from NTPC Vindhyachal, in three successive phases. Phase 1 optimises the TerraZyme dosage; Phase 2 introduces fly ash to the optimum TerraZyme-treated soil; Phase 3 measures swelling magnitude and swelling pressure per IS 2720 (Part 41) for the untreated, the optimum TerraZyme-treated, and the optimum combined soils. The scope and the acknowledged limitations of this programme are stated in Section 3.7 and Section 6.

2. MATERIALS

2.1 Black cotton soil

The black cotton soil (BCS) used in this study was collected from the Sports Complex field on the campus of Maulana Azad National Institute of Technology (MANIT), Bhopal, Madhya Pradesh, India. The soil was excavated to a depth of about 0.5 m below the ground surface to avoid root-zone and surface-contamination effects. The collected soil was air-dried, pulverized, and sieved through a 4.75 mm IS sieve before testing. Initial geotechnical characterization in accordance with the relevant sections of IS 2720 confirmed the soil as CH (clay of high compressibility and plasticity) per the Unified Soil Classification System (USCS). The index and engineering properties of the untreated BCS are summarized in Table 1.

The liquid limit of the soil was 61% and the plastic limit was 23%, yielding a plasticity index of 38%. The free swell index (FSI), determined as per IS 2720 (Part 40)–1977, was 66.67%, exceeding the 50% threshold specified in the standard for classifying a soil as expansive, confirming the highly expansive nature of the material. The specific gravity was 2.67. Standard Proctor compaction (IS 2720 Part 7–1980) yielded a maximum dry density (MDD) of 1.44 g/cm³ at an optimum moisture content (OMC) of 20.68%. The unconfined compressive strength (UCS) of the untreated soil was 117.44 kN/m² on the day of preparation (Day 0) and 134.38 kN/m² after seven days of air curing. The soaked and unsoaked California Bearing Ratio (CBR) values of the untreated soil were 3.28% and 13.42%, respectively, both obtained per IS 2720 (Part 16)–1987. Wet sieve analysis indicated that 95% of the soil consisted of fine-grained material (passing 75 µm sieve), with only 5% coarse fraction.

The presence of montmorillonite in the soil mineral assemblage — inferred from the high plasticity index, free swell index, and liquid limit — is consistent with the characteristic behaviour of Deccan basalt-derived black cotton soils commonly encountered in central India (Nelson and Miller, 1992; Ranjan and Rao, 2016). It is emphasised that the mineralogy was inferred from index behaviour and was not verified by X-ray diffraction; the implications of this are discussed in Section 6.

2.2 Class-F fly ash

The fly ash used in this study is Class-F fly ash, procured from the Vindhyachal Super Thermal Power Station (NTPC), Madhya Pradesh. Class-F fly ash is derived from the combustion of bituminous and anthracite coal, and is characterized by a calcium oxide (CaO) content of less than 10%, giving it a primarily pozzolanic (rather than self-cementitious) character. It requires an activator — such as free lime or an alkaline environment — to initiate cementitious reactions (Noaman et al., 2022).

Table 1 Basic geotechnical properties of BCS and Class-F fly ash

Property	BCS	Fly ash
Coarse fraction (%)	5	29.77
Fine fraction, < 75 μm (%)	95	70.23
Liquid limit (%)	61	27
Plastic limit (%)	23	NP
Plasticity index (%)	38	—
Free swell index (%)	66.67	28.57
Specific gravity	2.67	1.96
MDD (g/cm^3)	1.44	—
OMC (%)	20.68	—
UCS Day 0 (kN/m^2)	117.44	—
UCS Day 7 (kN/m^2)	134.38	—
Soaked CBR (%)	3.28	—
Unsoaked CBR (%)	13.42	—
USCS classification	CH	ML

The basic properties of the fly ash were determined in the laboratory. The specific gravity was 1.96, significantly lower than that of the BCS (2.67), reflecting its hollow spherical particle morphology. The liquid limit was 27% and the material was non-plastic, classifying it as ML (silt of low plasticity and compressibility) under the USCS. The free swell index was 28.57%, well below the 50% threshold for expansive classification. Wet sieve analysis revealed 70.23% fine content and 29.77% coarse content. In the present study, fly ash was added at proportions of 10%, 20%, 30%, and 40% by dry weight of the BCS, based on the range documented in the literature for expansive soil stabilization with Class-F fly ash (Phanikumar and Sharma, 2007; Noaman et al., 2022). Two properties of this material are critical to the interpretation of the results that follow: it is non-plastic, so it contributes no cohesion to the mix, and it is not self-cementing in the absence of an alkaline activator, so it contributes no cementitious bonding either.

2.3 TerraZyme

TerraZyme (TZ) is a non-toxic, non-corrosive liquid bio-enzyme concentrate derived from the fermentation of vegetable and fruit extracts, procured from M/s Avijeet Agencies Pvt. Ltd., Chennai (manufactured by Nature Plus). Its principal characteristics include a pH of 4.3–4.6, specific gravity of 1.02–1.10, CEC of 3.87, and complete water solubility. TerraZyme operates on the principle of ionic catalysis: enzyme molecules associate with the negatively charged clay surfaces, reducing the electrical double-layer thickness, which permanently lowers water affinity, plasticity, and void ratio, and improves compaction (Gupta et al., 2017). Four dosages (40, 67, 100, and 133 ml/m^3) were investigated to determine the performance-based optimum for BCS, rather than relying on the generic manufacturer recommendation (approximately 90 ml/m^3). TerraZyme has been accredited by the Indian Roads Congress for

subgrade stabilization (IRC, 2022) and has been used in several Indian states and overseas. The acidic pH of the concentrate is noted here because it becomes significant in Section 4.8: an acidic admixture cannot supply the alkaline environment that Class-F fly ash requires for pozzolanic activation.

3. EXPERIMENTAL PROGRAMME

3.1 Structure of the experimental programme

The experimental study was organised into three successive phases. A flowchart of the programme is given in Figure 1. The characterisation of the individual materials, reported in Section 2, is treated as preliminary work and is not counted as a phase.

In Phase 1, BCS was treated with TerraZyme at four dosages: 40, 67, 100, and 133 ml/m^3 . At each dosage the following tests were performed: Atterberg limits (liquid limit and plastic limit), standard Proctor compaction, UCS at Day 0 and Day 7, and soaked and unsoaked CBR. The optimum TerraZyme dosage was identified as the level corresponding to the maximum improvement in UCS and soaked CBR.

In Phase 2, fly ash was added at 10%, 20%, 30%, and 40% by dry weight of the soil to BCS previously mixed with the optimum TerraZyme dosage from Phase 1. Each fly ash proportion was retested with the same set of tests — Atterberg limits, compaction, UCS (Day 0 and Day 7), and soaked/unsoaked CBR — to identify the best-performing fly ash content in the combined system.

In Phase 3, the swelling magnitude and swelling pressure were determined for three selected mixes: untreated BCS, BCS with optimum TerraZyme alone, and BCS with optimum TerraZyme combined with the selected fly ash content. This phase was designed to evaluate the effectiveness of the combined treatment in addressing volumetric instability, the principal engineering concern when BCS is used as a road subgrade.

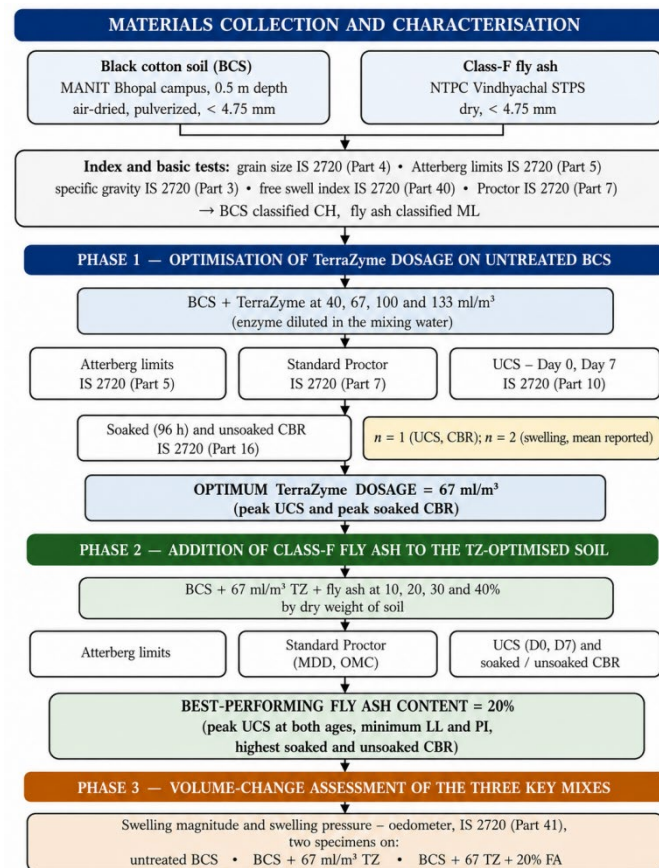


Figure 1 Flowchart of the three-phase experimental programme

3.2 Sample preparation and conversion of the volumetric TerraZyme dosage

All soil samples were prepared by first thoroughly air-drying the collected BCS, breaking up clods, and passing the material through a 4.75 mm IS sieve. For fly ash mixes, the fly ash was dry-blended with the soil before the enzyme solution was introduced, so that the pozzolanic component was uniformly distributed through the matrix before wetting.

The TerraZyme dosage is specified by the manufacturer per unit volume of *compacted* soil (ml/m^3). Because the laboratory specimens are small, the concentrate volume required for a single specimen is of the order of microlitres and cannot be measured directly with acceptable accuracy. The following procedure was therefore adopted and is reported here in full so that the work can be reproduced. (i) The dry mass of soil required to fill each mould at the MDD of the mix concerned was computed from the mould volume. (ii) The volume of TerraZyme concentrate required was

obtained as the product of the dosage ($67 \text{ ml}/\text{m}^3$ at the optimum) and the compacted volume of the batch. (iii) A stock solution was prepared by diluting 10 ml of TerraZyme concentrate to 1000 ml with distilled water (1:100 by volume). (iv) The required volume of stock solution, one hundred times the concentrate volume, was measured with a 25 ml graduated burette (least count 0.05 ml) and made up with additional distilled water to the total mass of water corresponding to the OMC of that mix. (v) The whole of this liquid was added to the dry soil (or soil-fly ash blend) in a mechanical mixer and mixed for five minutes. The specimens were compacted immediately after mixing.

Table 2 gives the resulting quantities for each mould used in this study. For direct comparison with studies that dose the enzyme per unit mass of soil, the optimum dosage of $67 \text{ ml}/\text{m}^3$ corresponds to $67 \div 1520 = 0.044 \text{ ml}$ per kilogram of dry soil at the MDD of the TZ-optimised mix ($1.52 \text{ g}/\text{cm}^3$), or $0.047 \text{ ml}/\text{kg}$ at the MDD of the untreated soil ($1.44 \text{ g}/\text{cm}^3$).

Table 2 Conversion of the volumetric TerraZyme dosage ($67 \text{ ml}/\text{m}^3$) to the quantity added per specimen

Test / mould	Mould dimensions (mm)	Mould volume ($\times 10^{-5} \text{ m}^3$)	TZ concentrate per specimen (μL)	1:100 stock solution per specimen (mL)
UCS specimen	38 ϕ $\times$ 76 h	8.62	5.8	0.58
Standard Proctor mould	100 ϕ $\times$ 127.3 h	100.0	67.0	6.70
CBR mould	150 ϕ $\times$ 175 h	309.3	207.2	20.72
Oedometer (swelling) ring	60 ϕ $\times$ 20 h	5.65	3.8	0.38

3.3 UCS specimen preparation

Cylindrical specimens for the UCS test were prepared in a split mould 38 mm in diameter and 76 mm in height (length-to-diameter ratio = 2:1), in accordance with IS 2720 (Part 10)–1991. The soil

mixture was compacted into the mould in three equal layers using a standard rammer at the OMC and to the MDD determined from the standard Proctor test for each mix. Specimens were prepared in two sets per mix: one set was tested immediately upon preparation (Day 0), and the second set was sealed in polythene bags to prevent

moisture loss and cured at ambient laboratory temperature for seven days before testing (Day 7). UCS tests were conducted in a strain-controlled loading frame at a strain rate of 1.25 mm/min, and the peak deviator stress was recorded as the unconfined compressive strength.

3.4 CBR specimen preparation

CBR specimens were prepared by static compaction of the soil mixture in a standard CBR mould with an internal diameter of 150 mm and height of 175 mm, compacted in five equal layers per IS 2720 (Part 16)–1987. Each layer was compacted using a 2.6 kg rammer dropped from a height of 310 mm. Specimens were compacted at the OMC of the respective mix to achieve the corresponding MDD. After compaction, specimens were soaked in water for 96 hours (four days) with a 4.54 kg annular surcharge weight placed on top to simulate field overburden conditions, and the soaked CBR was recorded at 2.5 mm penetration. Unsoaked CBR was measured on separate companion specimens that were never soaked, so as to assess the immediate bearing capacity of each mix.

3.5 Swelling and swelling-pressure specimen preparation

Swelling pressure was determined using the consolidation (constant-volume) method specified in IS 2720 (Part 41)–1977. Disc-shaped specimens 60 mm in diameter and 20 mm thick (diameter-to-thickness ratio ≥ 3 , as stipulated in the standard) were prepared by static compaction at the OMC and MDD of the respective mix. Two specimens were prepared per mix variation and the reported values are the mean of the two. The specimens were mounted in a standard oedometer and inundated with distilled water. The load required to maintain the specimen at its initial height was recorded at equilibrium, and the swelling pressure was computed as this load divided by the cross-sectional area of the specimen. The test was continued until no further change in load was observed over a 24-hour period. Swelling magnitude was recorded on companion specimens of the same initial thickness under the free-swell (zero surcharge) condition, using a dial gauge of 0.002 mm least count, and is reported both in millimetres and as a percentage of the 20 mm initial specimen thickness (Table 4).

3.6 Replication, repeatability and data reliability

The number of specimens tested is stated here explicitly. The liquid limit was determined twice on the untreated soil and once on each treated mix; the plastic limit, the plasticity index and the standard Proctor compaction curve were determined once per mix; the unconfined compressive strength was measured on one specimen per mix at each of the two curing ages; the California bearing ratio was determined once per mix in each of the soaked and the unsoaked conditions; and the swelling tests were carried out on two companion specimens per mix, the reported value being the mean of the two. Replicate UCS and CBR specimens were therefore not tested, and no standard deviation or confidence interval can be quoted for the strength and bearing-capacity values in Table 3. This is a limitation of the study, and it is restated in Section 6. Specimens whose compacted dry density departed from the target MDD of the mix by more than 1% were rejected and remoulded, so that preparation variability is controlled even though it is not quantified.

Three considerations bear on the weight that single-point values can carry. First, the differences on which the conclusions rest are far larger than the repeatability normally reported for these test methods: the Day-7 UCS of the TerraZyme-optimised mix is nine times that of the untreated soil, and the soaked CBR of every combined mix is less than a fifth of the TerraZyme-only value. Second, every mix was prepared from a single homogenised batch of the same bulk soil sample, so between-batch material variability does not confound the comparison between mixes. Third, and most importantly, seven independent measurements — liquid limit, plasticity index, MDD, OMC, UCS at two curing ages, and CBR in two conditions — all locate the same optimum at 67 ml/m³ and all reverse beyond it. A measurement artefact in any one test would not

reproduce itself across seven different test methods, and this internal consistency is the principal evidence that the trends reported here are real. It is not a substitute for replication, and is recommended as further work.

3.7 Test standards and scope of the programme

All tests were performed in the Geotechnical Engineering Laboratory, Department of Civil Engineering, MANIT Bhopal, in accordance with Bureau of Indian Standards guidelines. Liquid and plastic limits were determined per IS 2720 (Part 5)–1985, particle size distribution per IS 2720 (Part 4)–1985, free swell index per IS 2720 (Part 40)–1977, specific gravity per IS 2720 (Part 3)–1980, compaction (MDD and OMC) per IS 2720 (Part 7)–1980, UCS per IS 2720 (Part 10)–1991, CBR per IS 2720 (Part 16)–1987, and swelling pressure per IS 2720 (Part 41)–1977.

The programme is deliberately confined to the macro-scale index, compaction, strength, bearing-capacity and volume-change properties that govern the design of a stabilised subgrade or cover layer. Microstructural and mineralogical techniques (SEM, XRD, FTIR), suction measurement, permeability and wet-dry durability testing were not available at the host institution during this work and were therefore outside the scope of the programme. The mechanistic interpretation offered in Section 4.8 is accordingly built from the macro-scale evidence — the plasticity, compaction, strength, bearing-capacity and swelling data, and derived indices such as the soaked-to-unsoaked CBR ratio — and is presented as a physically consistent conceptual model rather than as directly observed microstructure. The full set of limitations and the recommended follow-up programme are given in Section 6.

4. RESULTS AND DISCUSSION

4.1 Basic properties of the materials

The geotechnical properties of the BCS and Class-F fly ash are summarized in Table 1. The BCS had a liquid limit of 61%, plasticity index of 38%, and free swell index of 66.67% — exceeding the IS 2720 (Part 40) threshold of 50% for expansive soils (Nelson and Miller, 1992). The soaked CBR of 3.28% is well below the IRC minimum of 5% for a road subgrade, confirming the need for stabilization. The fly ash was non-plastic with an FSI of 28.57%, classified as ML under the USCS.

4.2 Effect on index properties

The Atterberg limit results for both phases are presented in Figure 2 and Table 3. In Phase 1, TerraZyme progressively reduced the liquid limit (LL) from 61% to a minimum of 49% and the plasticity index (PI) from 38% to 23% at the optimum dosage of 67 ml/m³. The increase in both LL and PI beyond 67 ml/m³ confirmed the presence of an inverted-V optimum. This behaviour is consistent with the electrochemical double-layer compression mechanism of TerraZyme: the enzyme occupies the available clay surface exchange sites at the optimum dosage, but beyond this point the surplus enzyme is not adsorbed and acts as a lubricating pore fluid rather than as an exchanger (Gupta et al., 2017; Agarwal and Kaur, 2014; Muguda and Nagaraj, 2019).

In Phase 2, as fly ash was added to the TZ-treated soil (67 ml/m³), the LL increased slightly at 10% FA (due to the water absorption capacity of Class-F fly ash), but at 20% FA the absorption effect was offset and the LL reached a minimum of 45% (PI = 28%). The net decrease in PI from 38% (untreated) to 28% (67 ml/m³ TZ + 20% FA) represents a 26% reduction, attributable to the combined action of TerraZyme reducing clay–water affinity and fly ash physically diluting the expansive clay fraction (Phanikumar and Sharma, 2007; Noaman et al., 2022). It is important to note that the PI of the combined mix (28%) is higher than that of the TerraZyme-only optimum (23%): on plasticity criteria the fly ash addition is a partial reversal of the improvement achieved by the enzyme alone, not an addition to it. The mix nevertheless remains classified CH, so neither treatment removes the soil from the high-plasticity category.

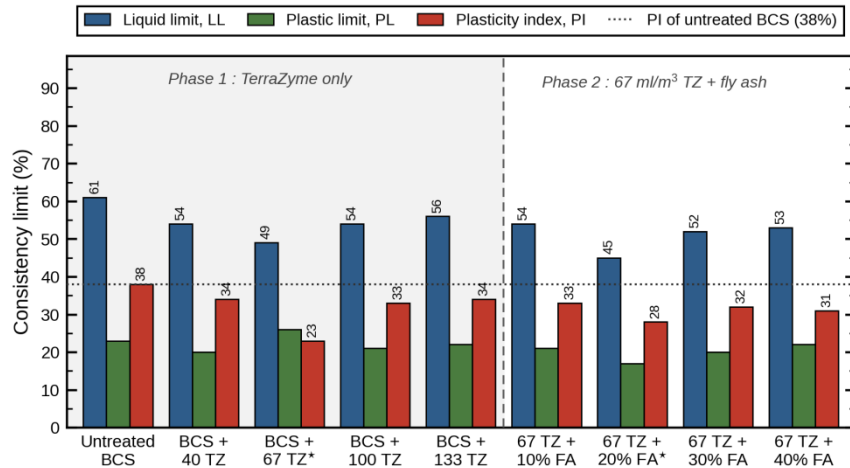


Figure 2 Liquid limit, plastic limit and plasticity index for all nine mix variations. The dotted line marks the plasticity index of the untreated soil

Table 3 Consolidated results for all mix variations (one specimen per mix per condition, Section 3.6; ★ = selected mix; D0 = Day 0; D7 = Day 7)

Mix	LL (%)	PI (%)	MDD (g/cm ³)	OMC (%)	UCS D0 (kN/m ²)	UCS D7 (kN/m ²)	Soaked CBR (%)	Unsoaked CBR (%)	CBR ratio soaked/unsoaked
Original BCS	61	38	1.44	20.68	117.44	134.38	3.28	13.42	0.24
+ 40 ml/m ³ TZ	54	34	1.43	17.36	210.15	440.30	10.58	13.87	0.76
+ 67 ml/m ³ TZ ★	49	23	1.52	13.34	838.24	1205.43	13.87	16.24	0.85
+ 100 ml/m ³ TZ	54	33	1.41	24.19	659.82	889.43	10.49	14.23	0.74
+ 133 ml/m ³ TZ	56	34	1.40	24.41	650.50	715.50	6.57	10.04	0.65
67 TZ + 10% FA	54	33	1.40	20.75	207.57	340.72	2.20	7.52	0.29
67 TZ + 20% FA ★	45	28	1.40	18.84	292.54	366.55	2.55	9.12	0.28
67 TZ + 30% FA	52	32	1.40	15.99	206.66	270.20	2.37	8.03	0.30
67 TZ + 40% FA	53	31	1.31	19.70	170.64	219.70	1.82	6.37	0.29

4.3 Effect on compaction characteristics

In Phase 1, the MDD increased from 1.44 g/cm³ (untreated) to a maximum of 1.52 g/cm³ at 67 ml/m³ TZ (+5.6%), and the OMC decreased from 20.68% to 13.34% (−35.5%). Both MDD and OMC reverted toward the untreated values beyond 67 ml/m³ TZ, consistent with surplus enzyme acting as a lubricant. The direct field advantage of the reduced OMC at the optimum dosage is that less water is required for compaction, lowering construction cost in water-scarce regions.

In Phase 2, all TZ + FA mixes gave MDD values in the range 1.31–1.40 g/cm³, lower than both the untreated BCS and the TZ-only optimum, owing to the low specific gravity of the fly ash (1.96 against 2.67 for the BCS). The MDD values obtained at 10%, 20% and 30% fly ash are indistinguishable from one another within the resolution of the standard Proctor test, and they are therefore reported to two decimal places (1.40 g/cm³ in each case) throughout Table 3, consistently with the remainder of the table.

The OMC of the Phase-2 mixes did not vary monotonically with fly ash content, and the lowest OMC recorded in Phase 2 was 15.99% at 30% fly ash, not 18.84% at 20% fly ash. The compaction

results do not, by themselves, identify an optimum fly ash content: MDD is essentially insensitive to fly ash content between 10% and 30% and falls only at 40%, while OMC scatters within a narrow band without a clear trend. Compaction was accordingly *not* used as a selection criterion.

The 20% fly ash content was selected on strength and plasticity criteria, which are stated here explicitly. Among the four fly ash proportions tested, 20% simultaneously gave (i) the highest UCS at Day 0 (292.54 kN/m²) and at Day 7 (366.55 kN/m²), (ii) the lowest liquid limit (45%) and the lowest plasticity index (28%), and (iii) the highest soaked CBR (2.55%), and (iv) the highest unsoaked CBR (9.12%). It is the only fly ash proportion that is simultaneously best on all three of these criteria. It did not give the highest unsoaked CBR, which occurred at 10% fly ash (9.12%); this exception is discussed in Section 4.5. Because the selection is made on strength and plasticity rather than on compaction, and because — as Section 4.5 shows — none of the fly ash proportions produces an acceptable bearing capacity, the 20% content is described in the present study as the best-performing fly ash proportion within the range tested rather than as an optimum in the design sense.

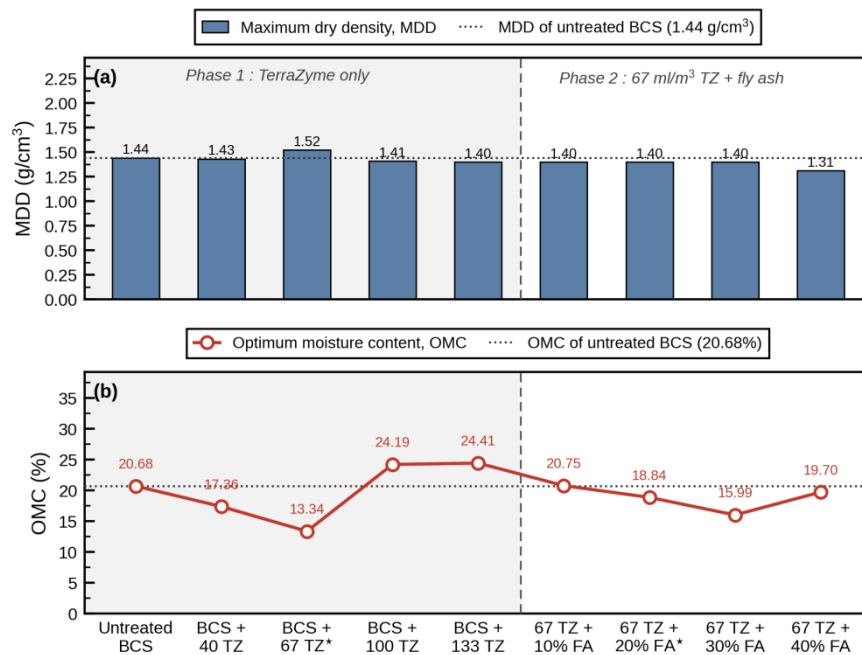


Figure 3 (a) Maximum dry density and (b) optimum moisture content for all nine mix variations. The dotted lines mark the values for the untreated soil. Note that the minimum OMC in Phase 2 occurs at 30% fly ash, not at 20%

4.4 Effect on unconfined compressive strength

Figure 4 and Table 3 present the UCS results for both phases. In Phase 1, the UCS at 67 ml/m³ TZ reached 838.24 kN/m² (Day 0) and 1205.43 kN/m² (Day 7) — approximately 7.1× and 9.0× the untreated values, respectively. This increase results from TerraZyme catalysing cation-exchange reactions at clay particle interfaces, reducing the void ratio, expelling pore water, and forming strong inter-particle bonds (Kushwaha et al., 2018; Kolhe and Dhatrik, 2022). The upward trend between Day 0 and Day 7 at all dosages confirms a time-dependent post-compaction strengthening mechanism consistent with enzymatic curing. Shukla et al. (2003) achieved UCS improvements of 65–252% after four weeks of bio-enzyme treatment; the larger improvement observed here (797% at Day 7) is attributed to the high montmorillonite content and 95% fines fraction of the MANIT BCS, which provides a large reactive surface area, and to the very low untreated baseline strength.

In Phase 2, UCS values for all TZ + FA mixes were substantially lower than the TZ-only peak; the 20% FA mix yielded the highest values, with Day 0 UCS of 292.54 kN/m² and Day 7 UCS of 366.55 kN/m². Expressed against the TerraZyme-only optimum this is a reduction of 65% at Day 0 and 70% at Day 7; expressed against the untreated soil it remains an improvement of 2.5× and 2.7× respectively. The UCS of the combined mix therefore does lie between the untreated soil and the TerraZyme-only mix, which is the behaviour expected of a genuinely intermediate material. As Section 4.5 shows, the CBR does not. The loss of strength relative to TZ alone is chemically expected: Class-F fly ash in the absence of an alkaline activator does not add cementitious strength, and its contribution to this system is purely physical — dilution of the clay fraction and inter-aggregate void filling (Noaman et al., 2022). The persistence of the Day 0 to Day 7 strength gain in the combined mixes indicates that TerraZyme curing continues in the presence of fly ash, although at a reduced absolute level.

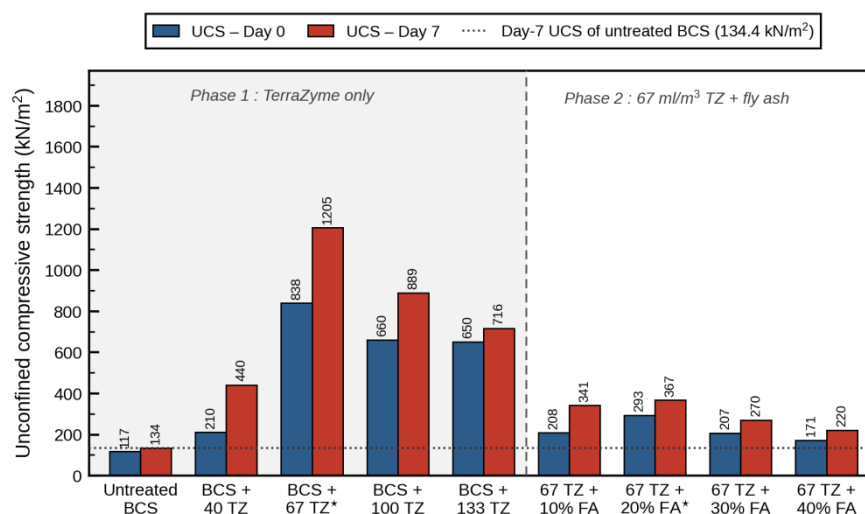


Figure 4 Unconfined compressive strength at Day 0 and Day 7 for all nine mix variations (one specimen per mix per curing age; see Section 3.6)

4.5 Effect on California bearing ratio

CBR results are shown in Figure 5 and Table 3. In Phase 1, the soaked CBR increased from 3.28% (untreated) to 13.87% at 67 ml/m³ TZ — a 4.2-fold improvement that places the treated soil well above the IRC minimum subgrade requirement of 5% — while the unsoaked CBR rose only from 13.42% to 16.24%, an increase of 21%. The contrast between these two figures is itself the most informative result of Phase 1 and is developed in Section 4.8: the enzyme does comparatively little to the dry bearing capacity of the soil and a great deal to its saturated bearing capacity, which is the signature of a treatment that works by excluding water rather than by cementing particles. The improvement is attributed to the hydrophobic coating formed by TerraZyme on the clay surfaces through cation exchange, which inhibits moisture ingress and prevents the swelling-induced strength loss characteristic of untreated montmorillonitic soils (Rajoria and Kaur, 2014; Panchal et al., 2017).

In Phase 2, this picture reverses completely. Adding fly ash to the TerraZyme-treated soil reduced the soaked CBR to 1.82–2.55% and the unsoaked CBR to 6.37–9.12%. These are not merely reductions relative to the TerraZyme-only optimum: both the soaked and the unsoaked CBR of every TZ + FA mix are lower than the corresponding values of the natural, untreated soil. At the best-performing fly ash content of 20%, the soaked CBR is 22% below the untreated value (2.55% against 3.28%) and the unsoaked CBR is 32% below it (9.12% against 13.42%). On bearing-capacity criteria the combined treatment is therefore worse than leaving the soil untreated.

Water absorption during soaking is one contributing mechanism, but it is not sufficient. The fly ash during the 96-hour soaking period

improved subgrade drainage as the mitigation. That explanation is retained here as one contributing mechanism (see Section 4.8), but it is not sufficient, and the drainage recommendation does not follow from it. The unsoaked CBR is measured on companion specimens that are never soaked, and it too has fallen sharply — from 16.24% for the TerraZyme-only optimum to 9.12% at 20% fly ash, a reduction of 43.8%, and 32.0% below the untreated soil. A loss that is present in the unsoaked condition cannot be explained by water absorption during soaking and cannot be mitigated by drainage. The reduction in unsoaked CBR indicates a loss of the intrinsic penetration resistance of the compacted matrix — that is, a loss of the enzyme-generated cohesive bonding — caused by the fly ash itself. This is analysed in Section 4.8.

A useful derived quantity is the ratio of soaked to unsoaked CBR, which measures how much of a material's dry bearing capacity survives saturation and is therefore a direct index of moisture sensitivity. It is given in the last column of Table 3 and plotted in Figure 5(b). For the untreated BCS it is 0.24: the soil loses roughly three quarters of its bearing capacity on soaking. TerraZyme raises it to 0.65–0.85 across the dosage range tested, reaching 0.85 at the optimum of 67 ml/m³, so that the treated soil retains about six sevenths of its dry bearing capacity after 96 hours of soaking. This is the clearest single piece of evidence in the study that TerraZyme acts as a surface-coating, water-excluding agent rather than as a binder: a cementing agent would raise the unsoaked strength in proportion, and the unsoaked CBR rises by only 21%. Adding fly ash drives the ratio back down to 0.28–0.30, within 0.06 of the untreated value. The fly ash therefore does not merely dilute the strength of the enzyme-treated soil; it very nearly cancels the specific improvement that the enzyme was providing.

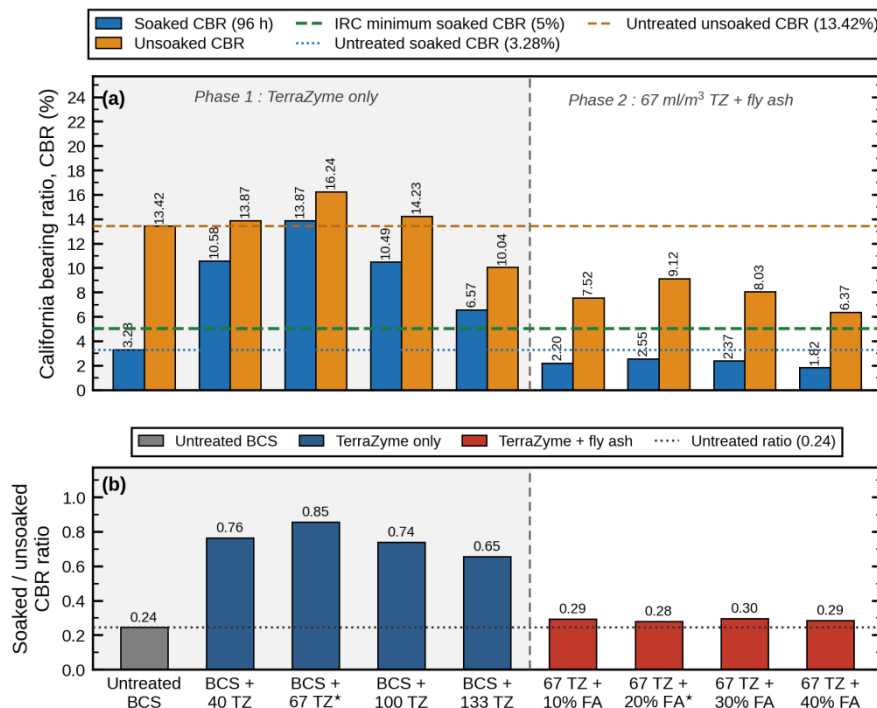


Figure 5 (a) Soaked (96 h) and unsoaked CBR for all nine mix variations; the dotted and dashed horizontal lines mark the soaked and unsoaked CBR of the untreated soil and the IRC minimum soaked CBR of 5%. Every TZ + FA mix falls below both untreated baselines. (b) Ratio of soaked to unsoaked CBR, an index of moisture sensitivity: TerraZyme raises it from 0.24 to 0.85, and fly ash returns it to 0.28–0.30. One specimen per mix in each condition; see Section 3.6.

4.6 Effect on swelling behaviour and swelling pressure

Table 4 and Figure 6 present the swelling data for the three key mixes. The untreated BCS reached a final swelling of 1.00 mm at equilibrium after approximately 300 hours, equal to 5.00% of the 20 mm initial specimen thickness. This slow, prolonged water adsorption is typical of montmorillonite-bearing expansive clays (Nelson and Miller, 1992). Treatment with 67 ml/m³ TZ reduced the final swelling to 0.87 mm (4.35%) and the swelling pressure from

2.40 to 2.05 kg/cm² (–14.6%), with equilibrium reached in about 210 hours. TerraZyme provides only partial disruption of the diffuse double layer; its primary contribution at the optimum dosage is strength enhancement rather than volumetric stability.

The greatest swelling control was achieved with the combined treatment (67 ml/m³ TZ + 20% FA): final swelling reduced to 0.50 mm (2.50% of the initial thickness, a 50% reduction relative to untreated) and swelling pressure to 1.35 kg/cm² (–43.8%), with equilibrium attained in less than 45 hours — an 85% reduction in

equilibration time. Two mechanisms contribute. The first is dilution: at 20% fly ash by dry weight the montmorillonitic clay content per unit dry mass falls by 20%, and the non-plastic silt-sized fly ash occupies inter-aggregate pore space that would otherwise be available for water uptake. The second is a density effect that must be acknowledged explicitly. The combined mix compacts to 1.40 g/cm³ against 1.52 g/cm³ for the TerraZyme-only mix and 1.44 g/cm³ for the untreated soil, and swelling pressure at constant volume is strongly dependent on compacted dry density. Part of the 43.8% reduction is therefore attributable to the lower density of the

compacted mix rather than to any change in the clay–water interaction. Phanikumar and Sharma (2007) made precisely this distinction, reporting a reduction of approximately 50% in swelling pressure at 20% fly ash when the comparison is made at constant dry unit weight, but an *increase* of approximately 60% when the comparison is made at constant weight of clay. The present result of –43.8% is measured at the natural compaction state of each mix and is therefore comparable to their constant-dry-unit-weight case; it should not be read as evidence that the clay itself has been rendered less expansive by a factor of two.

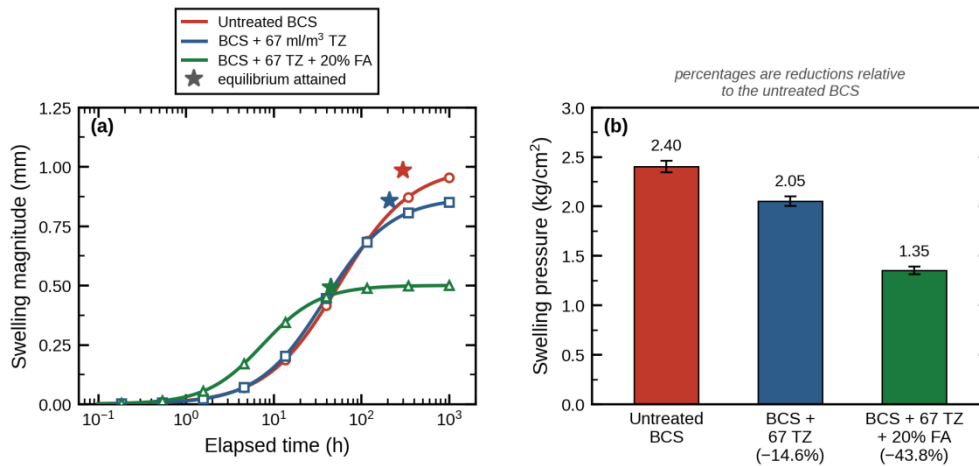


Figure 6 (a) Swelling magnitude against elapsed time on a logarithmic scale for the three key mixes; stars mark the attainment of equilibrium. (b) Swelling pressure of the three key mixes, with the percentage reduction relative to the untreated soil. Each value is the mean of two companion specimens (Section 3.6)

Table 4 Swelling results for the three key mixes (IS 2720 Part 41–1977; specimen thickness 20 mm; mean of two companion specimens)

Mix	Final swelling (mm)	Final swelling (% of initial thickness)	Time to equilibrium (h)	Swelling pressure (kg/cm ²)	Reduction in swelling pressure
Original BCS	1.00	5.00	300	2.40	—
BCS + 67 ml/m ³ TZ	0.87	4.35	210	2.05	–14.6%
BCS + 67 ml/m ³ TZ + 20% FA	0.50	2.50	45	1.35	–43.8%

4.7 Consolidated results and the strength–swelling trade-off

Figure 7 places all of the principal results on a common basis by normalising each measured quantity by the corresponding value for the untreated soil. For the properties with a lower value being better (panel (b)), both treated mixes are below unity for all four properties. The combined mix is superior to the other two mixes with respect to swelling magnitude (0.50 compared with 0.87), swelling pressure (0.56 compared with 0.85) and liquid limit (0.74 compared with 0.80); however, it is inferior to the other two mixes in terms of plasticity index (0.74 compared with 0.61). The plasticity index therefore increases as the liquid limit decreases between these two treated mixes; the non-plastic fly ash decreases the plastic limit from 26% to 17%, but does not significantly decrease the liquid limit from 49% to 45%. The reason why plasticity criteria only do not mark the combined mix as an improvement is consistent with the fly ash in this case being a non-plastic diluent, rather than a binder (Section 4.8c).

A treatment can reasonably be called balanced if its mechanical properties lie between those of the untreated soil and those of the best-performing single treatment, while its volume-change performance is superior to both. The combined TZ + FA mix satisfies this description for UCS and for volume change, but it fails it for bearing capacity, which is the property that governs pavement subgrade design.

4.8 Mechanistic interpretation: synergy in volume change, antagonism in strength

The results of Sections 4.2 to 4.7 are internally consistent only if TerraZyme and Class-F fly ash are recognised as acting on different phases of the soil fabric, with effects that are synergistic for volume change and antagonistic for strength. A conceptual model consistent with all of the macro-scale evidence is shown in Figure 8 and is developed below. Because microstructural verification was not available (Section 3.7), each element of the model is tied explicitly to the measured quantity that supports it.

(a) Action of TerraZyme. The enzyme is a large organic polyelectrolyte that adsorbs onto the negatively charged basal and edge surfaces of the montmorillonite platelets, displacing exchangeable inorganic cations and compressing the diffuse double layer. Four independent measurements support this. The plasticity index falls from 38% to 23%, which requires a reduction in the water held in the double layer. The OMC falls by 35.5% and the MDD rises by 5.6%, which requires the flocculated aggregates to pack more efficiently at a lower water content. The Day-0 to Day-7 UCS gain of 44% at the optimum dosage requires a time-dependent process, consistent with progressive adsorption and re-orientation rather than with instantaneous cementation. Most tellingly, the soaked/unsaturated CBR ratio rises from 0.24 to 0.85 while the unsaturated CBR itself rises by only 21%, which requires the treated fabric to resist saturation without being appreciably cemented — the

signature of a hydrophobic surface coating rather than of an inter-particle bond. The inverted-V response of every property beyond 67 ml/m³ requires a finite number of adsorption sites: once these are saturated, further enzyme remains in the pore fluid and acts as a

lubricant, which is exactly what the rising OMC (24.19% and 24.41% at 100 and 133 ml/m³) and falling MDD indicate.

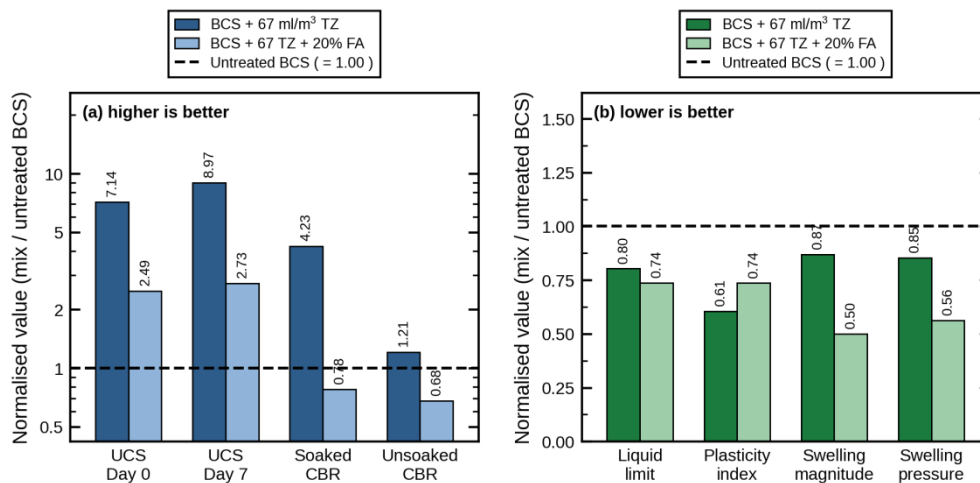


Figure 7 Performance of the two treated mixes normalised by the corresponding property of the untreated BCS: (a) properties for which a higher value is better, plotted on a logarithmic scale; (b) properties for which a lower value is better. The dashed line at unity represents the untreated soil

(b) Action of Class-F fly ash on volume change — the synergistic component. The fly ash reduces swelling by three physical routes and no chemical one. It dilutes the montmorillonitic fraction: at 20% addition the expansive clay content per unit dry mass falls by 20%. Its silt-sized, largely spherical particles occupy inter-aggregate pore space, reducing the volume available for water uptake and shortening the drainage path, which is why the time to equilibrium collapses from 300 to 45 hours. And the mix compacts to a lower dry density (1.40 against 1.52 g/cm³), which of itself lowers the constant-volume swelling pressure. The measured reduction of 43.8% in swelling pressure exceeds the 20% dilution, confirming that pore-filling and density effects contribute in addition to dilution, and the residual enzyme coating on the remaining clay continues to act. This is a genuine synergy in the sense that the combination outperforms either additive alone on volume change: TerraZyme alone achieved only -14.6%.

(c) Action of Class-F fly ash on strength and bearing capacity — the antagonistic component. Five mechanisms, acting together, account for the loss of both soaked and unsoaked CBR below the untreated values. They are set out in the order of their contribution as inferred from the data. First, and most importantly, the chemical incompatibility of the two additives. Class-F fly ash contains less than 10% CaO and is not self-cementing; it requires free lime or another source of alkalinity to dissolve its glassy aluminosilicate surface and form C-S-H. TerraZyme is acidic, with a pH of 4.3–4.6 (Section 2.3). Far from activating the fly ash, the enzyme lowers the pore-fluid pH and suppresses the very alkalinity on which pozzolanic reaction depends. The fly ash in this system is therefore chemically inert, and it is present purely as a non-plastic, non-cohesive filler. This is why the addition of fly ash produces no strength contribution to offset what it displaces, and it is the principal difference between an enzyme-fly ash system and the lime-fly ash systems for which the literature reports strength gains. Second, the loss of cohesive binder. In a fine-grained subgrade there is no granular interlock, and the resistance mobilised at 2.5 mm penetration in the CBR test is governed almost entirely by cohesion. Replacing 20% of an enzyme-bonded cohesive matrix with a non-plastic filler (PL = NP for the fly ash, Table 1) removes cohesion

without supplying friction in its place. This mechanism operates in the unsoaked as well as the soaked condition, and it is the reason the unsoaked CBR of the 20% fly ash mix falls by 43.8% relative to the TerraZyme-only optimum, from 16.24% to 9.12% — a result that water absorption during soaking cannot explain. Third, dilution of the enzyme itself. The dosage is fixed volumetrically at 67 ml/m³ of compacted mix, but a fifth of that volume is now occupied by fly ash whose glassy, low-charge surfaces offer no exchange sites. The enzyme is distributed over a larger total surface of which a smaller fraction is reactive clay, so the coating on the clay that remains is thinner and less complete. The soaked/unsoaked CBR ratio falling from 0.85 back to 0.28–0.30 is the direct evidence for this: the specific property that the enzyme was conferring, namely resistance to saturation, is the property that is lost. Fourth, the density penalty. The MDD of the combined mix is 7.9% lower than that of the TerraZyme-only mix. CBR is strongly dependent on compacted dry density, and a reduction of this magnitude alone would account for a substantial part of the loss in penetration resistance. Fifth, moisture ingress during soaking. The fly ash particles are porous and partly cenospheric, with a high water-holding capacity, and their surfaces are hydrophilic and — unlike the clay — not enzyme-coated. They therefore form continuous water pathways through what would otherwise be a water-repellent fabric, allowing the 96-hour soak to penetrate and soften the specimen. This mechanism is real but acts on the soaked condition only.

(d) Why 20% fly ash performs best among the fly ash contents. The behaviour in Phase 2 (20% highest UCS at both curing ages, lowest LL and PI, and highest soaked and unsoaked CBR) indicates that there are two competing effects that overlap around 20%. If fly ash content is below 20%, it will not fill the inter-aggregate pores and it will not provide any packing improvement. With above 20% the filler fraction becomes large enough to disrupt the continuity of the clay matrix and the strength decreases continuously until it reaches the lowest value at 40%. The reproducibility of each of the measured strength, bearing capacity and plasticity properties at the same fly ash content provides a single physical crossover, instead of unrelated optima. The untreated soil, whose bearing capacity is lost, can be restored by any of the no fly ash contents tested.

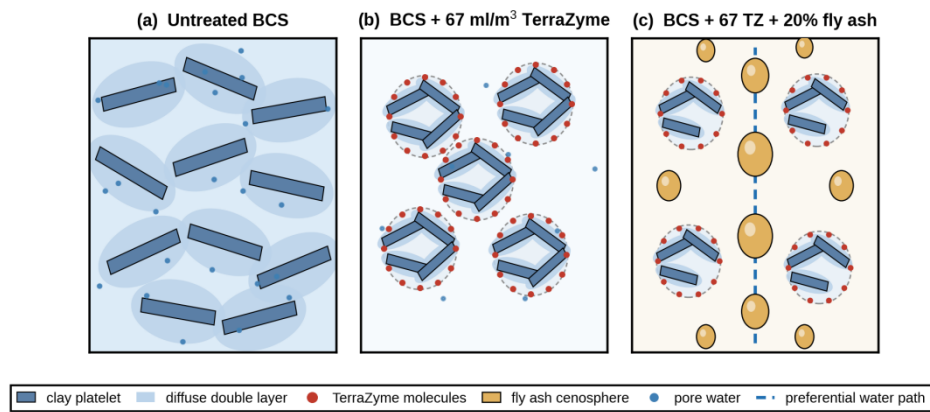


Figure 8 Conceptual model of the soil fabric. (a) Untreated BCS: dispersed platelets with a thick diffuse double layer, giving high plasticity, high swelling and low strength. (b) BCS + 67 ml/m³ TerraZyme: the enzyme coats the platelet surfaces, the double layer is compressed and the platelets flocculate into aggregates, giving peak UCS and peak CBR. (c) BCS + 67 ml/m³ TerraZyme + 20% Class-F fly ash: the cenospheres dilute the clay and fill inter-aggregate pores, reducing swelling, but they introduce uncoated hydrophilic surfaces that form a preferential water path and reduce the soaked CBR. The model is inferred from the macro-scale evidence discussed in Section 4.8 and is not a direct microstructural observation

4.9 Quantitative comparison with published studies

Table 5 compares the optimum dosages, strength gains and volume-change reductions obtained here with those reported in the studies most closely comparable to this work. Four differences require comment.

First, the optimum TerraZyme dosage found here (67 ml/m³, equivalent to 0.044 ml/kg) is between one and two orders of magnitude lower than several reported optima, which range from 0.05 to 0.80 ml/kg (Agarwal and Kaur, 2014) and up to several hundred ml/m³ (Panchal et al., 2017). Two causes are proposed. The soil used here has an exceptionally high fines content (95%) and free swell index (66.67%), so the exchange sites available to the enzyme are saturated at a comparatively low dosage; and the dosage is expressed per unit volume of compacted soil, so the relatively

high MDD of this material means that a given ml/m³ corresponds to a lower ml/kg than for a lighter soil. Reporting the dosage on both bases, as is done in Section 3.2, removes this ambiguity and is recommended for future studies.

Second, the UCS improvement of 797% at Day 7 exceeds the 65–252% of Shukla et al. (2003) and the approximately 200% of Agarwal and Kaur (2014). The principal cause is the very low untreated baseline of this soil (134.38 kN/m² at Day 7), which is roughly half the baseline reported in several of the comparison studies; the absolute treated strength of 1205 kN/m² is high but not exceptional. Percentage improvements in this field are strongly dependent on the untreated baseline and should not be compared without it.

Table 5 Quantitative comparison of the present results with published studies on bio-enzyme and fly ash stabilization

Study	Soil and stabilizer	Optimum dosage reported	Strength / bearing-capacity outcome	Plasticity and volume-change outcome
Present study, Phase 1	BCS (CH, LL 61, PI 38, FSI 66.67); TerraZyme	67 ml/m ³ ($\equiv$ 0.044 ml/kg at MDD)	UCS D0 +614%, UCS D7 +797%; soaked CBR 3.28 $\rightarrow$ 13.87% (+323%); unsoaked CBR 13.42 $\rightarrow$ 16.24% (+21%); soaked/unsoaked ratio 0.24 $\rightarrow$ 0.85	LL 61 $\rightarrow$ 49%; PI 38 $\rightarrow$ 23% (–39%); swelling pressure –14.6%
Present study, Phase 2	Same BCS; 67 ml/m ³ TZ + 20% Class-F fly ash	20% FA by dry weight	UCS D7 +173% vs untreated but –70% vs TZ alone; soaked CBR 2.55% (–22% vs untreated); unsoaked CBR 9.12% (–32% vs untreated); ratio back to 0.28	PI 38 $\rightarrow$ 28% (–26%); swelling magnitude –50%; swelling pressure –43.8%
Agarwal and Kaur (2014)	Black cotton soil; TerraZyme	0.25–4 ml per 5 kg of soil (0.05–0.80 ml/kg)	UCS increase of up to approximately 200% at 1 and 7 days	Not reported
Shukla et al. (2003)	Subgrade soils; bio-enzyme, laboratory and field	Manufacturer dosage	UCS improvement of 65–252% after four weeks	Reported to be cheaper than lime stabilization
Panchal et al. (2017)	Soil; TerraZyme	Diminishing returns beyond 400 ml/m ³	Progressive strength gain with curing period	Not reported
Aswathy et al. (2021)	Kaolinite (non-expansive); TerraZyme + lime	0.1 ml/kg	Best UCS obtained for the TerraZyme–lime mixture	Plasticity reduction reported
Kolhe and Dhatrak (2022)	Expansive BC soil; TerraZyme	Not comparable (per-mass basis)	7-day cured UCS substantially greater than immediate UCS	Not reported
Phanikumar and Sharma (2007)	Expansive CH clay; Class-F fly ash only	20% by dry weight	Not the focus of the study	Swell potential and swelling pressure reduced by $\approx$ 50% at constant dry unit weight, but increased by $\approx$ 60% at constant weight of clay
Das and Maharana (2019)	Soil; TerraZyme + fly ash	Combined mix reported	Improvement reported; soaked CBR not reported	Free swell index reduction reported

Third, the swelling pressure reduction of 43.8% at 20% fly ash is slightly smaller than the approximately 50% reported by Phanikumar and Sharma (2007) at the same fly ash content. This is consistent with the fact that in the present mixes the clay had already been partly stabilised by the enzyme, so a smaller further reduction remained available; and, as noted in Section 4.6, their reduction is quoted at constant dry unit weight.

Fourth, and most significantly, no previous combined bio-enzyme and fly ash study located by the authors reports soaked and unsoaked CBR for the same mixes against an untreated control. Das and Maharana (2019) and Raghwa and Sonthwal (2021) report improvements without a soaked CBR comparison against the untreated soil. This is the most likely reason that the antagonism documented here has not previously been reported, and it is a specific methodological recommendation of this paper that both CBR conditions be measured and reported against the untreated control in all future enzyme–filler studies. The advantage of the present scheme is that it isolates the fly ash effect at a fixed, independently optimised enzyme dosage; its limitation is that it is based on a single soil and a single fly ash source, so the numerical values should not be extrapolated to other expansive soils without confirmation.

4.10 Engineering implications and recommended field use

The practical consequences of the results are stated separately for the two treatments, because they are not interchangeable.

TerraZyme alone at 67 ml/m³ is the treatment recommended wherever bearing capacity governs. It raises the soaked CBR from 3.28% to 13.87%, comfortably above the IRC minimum of 5% for a subgrade, raises the Day-7 UCS nine-fold, reduces the plasticity index from 38% to 23%, reduces the compaction water demand by 35.5%, and produces a modest 14.6% reduction in swelling pressure. For a road subgrade on this soil it is an effective and economical treatment.

The combined treatment of 67 ml/m³ TerraZyme with 20% Class-F fly ash is not recommended as a load-bearing pavement subgrade. Its soaked CBR of 2.55% is below both the IRC minimum and the untreated soil, and its unsoaked CBR of 9.12% is about two thirds that of the untreated soil. A designer or contractor would not accept a bearing-capacity penalty of this size in exchange for a reduction in swelling, particularly when the reduction in swelling can be obtained by other means. The combination is, however, of genuine value where volume stability governs and the layer is not required to carry traffic loading: as a cushion or cover layer placed over an expansive subgrade, as a canal or pond lining, as a non-structural fill, or as a capping beneath a separately designed granular sub-base that carries the traffic load. In such applications the 43.8% reduction in swelling pressure, the 50% reduction in swelling magnitude, the 85% reduction in equilibration time, and the UCS of 366.55 kN/m² (2.7 times that of the untreated soil) are all beneficial, and the low CBR is not a controlling criterion.

Where both high bearing capacity and low swelling are required on the same soil, the results of this study point away from a single blended layer and towards either a layered solution — a TerraZyme-treated structural layer over a fly-ash-rich cushion — or towards replacing the Class-F fly ash with a lime-activated or Class-C fly ash system in which the pozzolanic reaction can proceed and can supply the cementation that the present combination lacks. Both options are outside the scope of the present programme and are recommended for future work.

5. CONCLUSIONS

This study investigated the stabilization of Black Cotton Soil (BCS) from the MANIT Bhopal campus using TerraZyme bio-enzyme and Class-F fly ash, individually and in combination, through a systematic three-phase experimental programme. Strength (UCS, soaked and unsoaked CBR), index (Atterberg limits), compaction and swelling characteristics were evaluated across nine mix variations, with the replicate scheme stated in full in Section 3.6. The following principal conclusions are drawn.

- (1) The optimum TerraZyme dosage for this BCS is 67 ml/m³, equivalent to 0.044 ml per kilogram of dry soil at the MDD of the treated mix. At this dosage the Day-0 UCS increased from 117.44 to 838.24 kN/m² (7.1×) and the Day-7 UCS from 134.38 to 1205.43 kN/m² (9.0×), the soaked CBR improved from 3.28% to 13.87% (4.2×) while the unsoaked CBR rose only from 13.42% to 16.24% (+21%). The MDD increased by 5.6% and the OMC decreased by 35.5%. Every property followed an inverted-V trend with dosage, confirming a well-defined optimum beyond which the surplus enzyme acts as a lubricant rather than as a stabilizer.
- (2) Within the combined system, 20% fly ash by dry weight was the best-performing of the four proportions tested. It was selected because it simultaneously gave the highest UCS at both curing ages, the lowest liquid limit and plasticity index, and the highest soaked CBR among the fly ash mixes. It was not selected on compaction grounds: the lowest OMC in Phase 2 occurred at 30% fly ash (15.99%), and the MDD is insensitive to fly ash content between 10% and 30%. At 20% fly ash the plasticity index reduced from 38% to 28%, the swelling pressure from 2.40 to 1.35 kg/cm² (−43.8%), the swelling magnitude by 50%, and the time to swelling equilibrium from 300 to 45 hours.
- (3) The addition of fly ash to the TerraZyme-treated soil is antagonistic to bearing capacity. Both the soaked CBR (1.82–2.55%) and the unsoaked CBR (6.37–9.12%) of every TZ + FA mix fell below the corresponding values of the untreated soil (3.28% and 13.42%). Because the loss is present in the unsoaked condition, it cannot be attributed to water absorption during soaking and cannot be mitigated by subgrade drainage. It is attributed principally to the chemical incompatibility of an acidic enzyme with a fly ash that requires an alkaline activator, and to the replacement of cohesive, enzyme-bonded clay by a non-plastic, chemically inert filler. The ratio of soaked to unsoaked CBR, which rises from 0.24 to 0.85 on treatment with TerraZyme alone, falls back to 0.28–0.30 when fly ash is added, showing that the fly ash very nearly cancels the specific benefit that the enzyme provides.
- (4) TerraZyme alone at 67 ml/m³ is the recommended treatment where bearing capacity governs. The TerraZyme plus 20% fly ash combination is recommended only where volume stability governs and the treated layer does not carry traffic loading — for example as an expansive-soil cushion or cover layer, a canal lining, or a non-structural fill.
- (5) The two additives act on different phases of the soil fabric. TerraZyme acts on the clay surfaces, compressing the diffuse double layer and conferring resistance to saturation; Class-F fly ash acts on the pore space and on the clay content, diluting the expansive fraction and filling inter-aggregate voids. The combination is synergistic for volume change, where the two mechanisms are additive, and antagonistic for strength, where the inert filler displaces the enzyme-bonded binder without replacing it. Recognising that the sign of the interaction depends on the property being measured is the principal contribution of this study, and it implies that enzyme–filler systems must be assessed against both strength and volume-change criteria before being recommended for field use.

6. LIMITATIONS AND SCOPE FOR FUTURE WORK

The limitations of this study are stated explicitly so that its conclusions are not extended beyond the evidence that supports them.

- (i) No microstructural or mineralogical characterisation was performed. Scanning electron microscopy, X-ray diffraction and Fourier-transform infrared spectroscopy were not available at the host institution during this work.

The montmorillonitic mineralogy of the soil is inferred from its index behaviour, and the fabric model of Section 4.8 is a conceptual construction consistent with the macro-scale data rather than a direct observation. Confirming the enzyme coating, the absence of pozzolanic reaction product, and the pore-filling role of the fly ash by SEM, XRD and FTIR is the highest priority for follow-up work, and the pore-fluid pH of the combined mix should be measured directly to test the chemical-incompatibility mechanism proposed in Section 4.8(c).

- (ii) The curing period was limited to seven days. The enzyme response is time-dependent and the literature reports continued strength gain at 28 days and beyond, so the long-term behaviour of both the TerraZyme-only and the combined mixes remains to be established.
- (iii) Durability was not assessed. Wetting–drying and, where relevant, freeze–thaw cycling are essential before any field recommendation, particularly for an enzyme whose action depends on a surface coating that may be progressively removed by cyclic saturation.
- (iv) Permeability, soil-water characteristic curves and suction measurements were not undertaken. Given that the mechanism proposed for the loss of soaked CBR involves preferential water pathways through uncoated fly ash surfaces, direct measurement of the hydraulic conductivity of the combined mix would be a decisive test of that mechanism.
- (v) Particle size distribution was determined for the parent materials only. The evolution of the aggregate size distribution with enzyme dosage and curing time, which would provide independent evidence of flocculation, was not tracked.
- (vi) A single soil and a single fly ash source were used, and all testing was at laboratory scale on remoulded specimens. The numerical optima reported here should be re-established for any other soil, and field-scale verification is required before implementation.

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