EMPLOYING UREASE-PRODUCING BACTERIA FROM THE HILLY REGIONS OF INDIA FOR MITIGATING RAINFALL-INDUCED SOIL EROSION

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Employing urease-producing bacteria from the hilly regions of India for mitigating rainfall-induced soil erosion

by

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CERTIFICATE

This is to certify that the thesis entitled "**EMPLOYING UREASE-PRODUCING BACTERIA FROM THE HILLY REGIONS OF INDIA FOR MITIGATING RAINFALL-INDUCED SOIL EROSION**" is being submitted by **Mr. Rituraj Devrani** in the fulfilment for the award of the degree of **Doctor of Philosophy** to the Indian Institute of Technology Delhi. This thesis is the record of the research work and is entirely carried out by him under my supervision and guidance. The research report and results presented in this thesis have not been submitted, in part or full, to any University or Institute for the award of any degree or diploma.

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ABSTRACT

Hilly regions worldwide are highly susceptible to geotechnical hazards such as landslides and soil erosion, particularly in weathered soils with weak or deteriorating strength. Addressing these challenges requires sustainable soil stabilization techniques that minimize environmental impact. Bio-cementation, particularly from microbial-induced calcite precipitation (MICP), has emerged as a promising alternative to conventional chemical soil stabilization methods. This technique harnesses urease-producing microorganisms to hydrolyze urea, generating carbonate ions that react with calcium to form calcium carbonate ($CaCO_3$) crystals. These precipitates enhance soil cohesion and structural integrity. However, the success of MICP depends on multiple factors, including bacterial strain selection, urease activity, chemical concentrations, and treatment methodologies. A deeper understanding of these parameters is essential for optimizing MICP effectiveness, particularly in erosion-prone hilly terrains.

This study focused on isolating and characterizing urease-producing bacterial strains from an erosion-prone region in India, where weak soil slopes frequently contribute to instability. The selected bacterial strains were assessed for urease activity, calcium carbonate precipitation potential, and genetic identity and were compared with the well-established ureolytic bacterium *Sporosarcina pasteurii* as a reference. The isolated bacterial strains exhibited superior urease activity and calcium carbonate precipitation capacity while maintaining an optimal alkaline environment crucial for effective MICP. These potent strains outperformed *S. pasteurii*, demonstrating their potential for site-specific bio-cementation applications.

To evaluate the efficacy of MICP for soil improvement, bio-cementation treatments were conducted in split-set molds, considering various treatment strategies such as treatment constituents and their sequence of addition, chemical substrate concentration, dosage intervals, solution volume, and treatment cycles. The effectiveness of the bio-cementation process was evaluated through unconfined compressive strength tests, pH monitoring, dry density

measurements, calcium carbonate quantification, and microstructural analysis. The results confirmed the successful formation of calcite crystals, which effectively bonded soil particles, leading to significant strength enhancement. The study demonstrated that the optimized treatment strategies had a direct impact on achieving the desired soil cementation and mechanical performance through a surface percolation method.

The endurance of bio-cemented soil was further assessed under multiple rainfall-drying cycles with varying raindrop kinetic energies and slope angles using a custom-built rainfall simulator. Untreated soil exhibited significant erosion under increasing slope gradients and rainfall energy, following a power law trend. In contrast, MICP-treated soil demonstrated remarkable resistance to erosion, maintaining structural stability even under extreme conditions. Bio-cementation effectively extended the time-to-ponding and reduced permeability, reinforcing soil strength and resilience. While surface resistance declined with prolonged exposure to intense rainfall, treatment with isolated bacterial strain consistently outperformed untreated and control and S. *pasteurii*-treated soils, showcasing improved erosion control and reduced structural degradation.

To bridge the gap between small-scale studies and real-world applications, an upscaled bio-cemented soil slope treated with isolated bacterial strains was evaluated under simulated rainfall conditions. This large-scale assessment provided more reliable insights into the erosion and hydrologic response of MICP-treated slopes, minimizing boundary condition effects that are often overlooked in smaller experimental setups. The impact of different MICP treatment durations on soil performance was also investigated, revealing that prolonged treatment resulted in enhanced surface erosion resistance. Comparative analysis of soil retention capacities and runoff characteristics between untreated and MICP-treated slopes confirmed that bio-cemented soils retained higher moisture content and experienced minimal erosion. These findings were further supported through microstructural and mineralogical investigations,

reinforcing the role of $CaCO_3$ precipitation in improving soil stability. Overall, this study establishes MICP as an effective and environmentally sustainable solution for mitigating rainfall-induced soil erosion in hilly regions. The superior performance of isolated bacterial strains highlights their potential for site-specific, offering an eco-friendly approach to enhancing geotechnical resilience. By integrating small-scale and upscaled experimental findings, this research provides critical insights into the practical application of bio-cementation for geotechnical resilience. These advancements pave the way for improved erosion control strategies and the development of sustainable infrastructure in vulnerable terrains.

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सारांश

विश्वभर के पहाड़ी क्षेत्र भू-तकनीकी खतरों, जैसे कि भूस्खलन और मिट्टी के कटाव, के प्रति अत्यधिक संवेदनशील होते हैं, विशेष रूप से उन मिट्टीयों में जो मौसम प्रभाव के कारण कमजोर या क्षरित हो जाती हैं। इन चुनौतियों का समाधान पर्यावरणीय प्रभाव को न्यूनतम रखते हुए स्थायी मिट्टी स्थिरीकरण तकनीकों के माध्यम से किया जा सकता है। बायो-सीमेंटेशन, विशेष रूप से माइक्रोबियल-इंड्यूस्ड कैल्साइट प्रीसिपिटेशन (एमआईसीपी), पारंपरिक रासायनिक स्थिरीकरण विधियों का एक आशाजनक विकल्प बनकर उभरा है। यह तकनीक यूरिया-उत्पादक सूक्ष्मजीवों का उपयोग करके यूरिया को हाइड्रोलाइज करती है, जिससे उत्पन्न कार्बोनेट आयन कैल्शियम के साथ अभिक्रिया करके कैल्शियम कार्बोनेट क्रिस्टल का निर्माण करते हैं। ये अवक्षेपण मिट्टी के कणों को आपस में जोड़कर उनकी संरचनात्मक अखंडता और स्थिरता को बढ़ाते हैं।

हालांकि, एमआईसीपी की प्रभावशीलता कई कारकों पर निर्भर करती है, जैसे कि बैक्टीरियल स्ट्रेन का चयन, यूरिएज़ एंजाइम की सक्रियता, रासायनिक सांद्रता, और उपचार की पद्धतियाँ। विशेष रूप से कटाव-प्रवण पहाड़ी क्षेत्रों में इस तकनीक को अनुकूलित करने के लिए इन कारकों की गहरी समझ आवश्यक है।

इस अध्ययन का उद्देश्य भारत के एक कटाव-प्रवण क्षेत्र से स्थानीय यूरिएज़-उत्पादक बैक्टीरियल स्ट्रेन्स को पृथक (आइसोलेट) करना और उनका चरित्रांकन करना था, जहाँ कमजोर मिट्टी ढलानों की अस्थिरता में योगदान देती है। चयनित बैक्टीरिया को यूरिएज़ गतिविधि, कैल्शियम कार्बोनेट अवक्षेपण क्षमता, और उनके आनुवंशिक पहचान के आधार पर परखा गया और उनकी तुलना *स्पोरोसारसीना पाश्चुरी* नामक एक प्रख्यात यूरोलाइटिक बैक्टीरिया से की गई। पृथक किए गए बैक्टीरियल स्ट्रेन्स ने उच्च यूरिएज़ गतिविधि और अधिक कैल्शियम कार्बोनेट अवक्षेपण क्षमता प्रदर्शित की, साथ ही इष्टतम क्षारीय वातावरण बनाए रखा, जो प्रभावी एमआईसीपी के लिए आवश्यक है। ये शक्तिशाली बैक्टीरियल स्ट्रेन्स

एस. *पाश्चुरी* से बेहतर प्रदर्शन करते पाए गए, जिससे उनके साइट-विशिष्ट बायो-सीमेंटेशन अनुप्रयोगों के लिए उपयुक्त होने की संभावना सिद्ध हुई।

मिट्टी सुधार के लिए एमआईसीपी की प्रभावशीलता का मूल्यांकन करने हेतु विभिन्न उपचार रणनीतियों का अध्ययन किया गया, जिनमें उपचार सामग्री के अनुक्रमण, रासायनिक पदार्थों की सांद्रता, खुराक अंतराल, घोल की मात्रा, और उपचार चक्र शामिल थे। बायो-सीमेंटेशन प्रक्रिया की प्रभावशीलता को अनकन्फाइंड कंप्रेसिव स्ट्रेंथ (यूसीएस) परीक्षण, पीएच-निगरानी, शुष्क घनत्व मापन, कैल्शियम कार्बोनेट मात्रांकन, और सूक्ष्म संरचनात्मक विश्लेषण के माध्यम से परखा गया। परिणामों से पुष्टि हुई कि कैल्साइट क्रिस्टल का सफलतापूर्वक निर्माण हुआ, जिसने मिट्टी के कणों को आपस में मजबूती से बाँध दिया, जिससे इसकी यांत्रिक शक्ति में उल्लेखनीय वृद्धि हुई। अध्ययन में यह पाया गया कि अनुकूलित उपचार रणनीतियों ने सतही परक्यूलेशन विधि के माध्यम से मिट्टी की मजबूती और स्थायित्व को बढ़ाने में महत्वपूर्ण भूमिका निभाई।

बायो-सीमेंटेड मिट्टी की टिकाऊपन को वर्षा-सुखाने चक्रों के तहत विभिन्न वर्षा ऊर्जा स्तरों और ढलान कोणों के साथ विशेष रूप से डिज़ाइन किए गए वर्षा सिमुलेटर का उपयोग करके परखा गया। असुधारित मिट्टी में जैसे-जैसे ढलान और वर्षा की तीव्रता बढ़ी, उसका कटाव तेजी से हुआ, जो एक पावर लॉ प्रवृत्ति का अनुसरण करता था। इसके विपरीत, एमआईसीपी-उपचारित मिट्टी ने उत्कृष्ट कटाव-प्रतिरोध प्रदर्शित किया और अत्यधिक प्रतिकूल परिस्थितियों में भी संरचनात्मक स्थिरता बनाए रखी। बायो-सीमेंटेशन ने पानी अवशोषण को बढ़ाया और मिट्टी की पारगम्यता को कम किया, जिससे मिट्टी की मजबूती और लचीलापन बढ़ा। हालांकि, दीर्घकालिक अत्यधिक वर्षा के संपर्क में आने से सतह की प्रतिरोधक क्षमता में गिरावट आई, लेकिन स्थानीय बैक्टीरियल स्ट्रेन द्वारा उपचारित मिट्टी ने *एस. पाश्चुरी*-उपचारित और असुधारित मिट्टी की तुलना में बेहतर प्रदर्शन किया, जिससे संरचनात्मक गिरावट में कमी और बेहतर कटाव नियंत्रण देखने को मिला।

छोटे पैमाने पर किए गए अध्ययनों और वास्तविक परिस्थितियों में इसके व्यावहारिक अनुप्रयोगों के बीच अंतर को कम करने के लिए, एक बड़ी बायो-सीमेंटेड मिट्टी ढलान को पृथक किए गए बैक्टीरियल स्ट्रेन्स से उपचारित करके नकली वर्षा परिस्थितियों के तहत परखा गया। इस बड़े पैमाने के परीक्षण से एमआईसीपी-उपचारित ढलानों की कटाव और जलगतिकी प्रतिक्रिया की अधिक सटीक जानकारी प्राप्त हुई, जिससे छोटे प्रयोगों में मौजूद सीमाएँ कम हो गईं। अलग-अलग एमआईसीपी उपचार अवधि का भी अध्ययन किया गया, जिससे पता चला कि दीर्घकालिक उपचार ने सतह के कटाव-प्रतिरोध को बढ़ा दिया।

एमआईसीपी-उपचारित और असुधारित ढलानों के बीच मिट्टी धारण क्षमता और अपवाह विशेषताओं की तुलनात्मक विश्लेषण से यह निष्कर्ष निकला कि बायो-सीमेंटेड मिट्टी में अधिक नमी बनी रही और कटाव न्यूनतम रहा। इन निष्कर्षों को सूक्ष्म संरचनात्मक और खनिजीय विश्लेषणों द्वारा और अधिक प्रमाणित किया गया, जिससे कैल्शियम कार्बोनेट अवक्षेपण की भूमिका मिट्टी की स्थिरता सुधारने में स्पष्ट हुई। समग्र रूप से, यह अध्ययन एमआईसीपी को वर्षा-प्रेरित मिट्टी कटाव को कम करने के लिए एक प्रभावी और पर्यावरणीय रूप से स्थायी समाधान के रूप में स्थापित करता है। स्थानीय बैक्टीरियल स्ट्रेन्स की उच्च प्रभावशीलता उनके साइट-विशिष्ट अनुप्रयोगों की क्षमता को उजागर करती है, जो भू-तकनीकी स्थिरता बढ़ाने के लिए एक पर्यावरण-अनुकूल विकल्प प्रदान करता है। छोटे और बड़े पैमाने के प्रयोगात्मक निष्कर्षों को एकीकृत करके, यह शोध बायो-सीमेंटेशन के व्यावहारिक अनुप्रयोगों पर महत्वपूर्ण अंतर्दृष्टि प्रदान करता है। ये उन्नतियाँ बेहतर कटाव नियंत्रण रणनीतियों और कमजोर भूभागों में सतत् अवसंरचना के विकास का मार्ग प्रशस्त करती हैं।

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MICP	Microbial-induced calcite precipitation
sp.	Species
º	Degree
$CaCO_3$	Calcium carbonate
$CO(NH_2)_2$	Urea
H_2O	Water
NH_2COOH	Carbamic acid
NH_3	Ammonia
H_2CO_3	Carbonic acid
H^+	Hydrogen ion
CO_3^{2-}	Carbonate ion
NH^+	Ammonium ion
OH^-	Hydroxide
K_{sp}	Solubility product constant
Ca^{2+}	Calcium ion
Cl^-	Chloride ion
HCO^{3-}	Bicarbonate ion
NH_4Cl	Ammonium chloride
°C	Degree Celsius
KCTC	Korean Collection for Type Cultures
$CaCl_2$	Calcium chloride
UCS	Unconfined compressive strength
OD_{600}	Optical density at 600 nm wavelength
ATCC	American Type Culture Collection

w/w	Weight/weight ratio
USCS	Unified Soil Classification System
SC	Clayey sand
DSMZ	Deutsche Sammlung von Mikroorganismen und Zellkulturen
PTCC	Pasteur Type Culture Collection
CGMCC	China General Microbiological Culture Collection Center
EPS	Extracellular polymeric substances
NB	Nutrient broth
NCIM	National Collection of Industrial Microorganisms
h	hour
w/w	Weight/weight ratio
w/v	Weight/volume ratio
ppm	parts per million
DNA	Deoxyribonucleic acid
PCR	Polymerase Chain Reaction
rRNA	Ribosomal Ribonucleic Acid
SEM	Scanning Electron Microscopy
XRD	X-ray diffraction
TS	Treatment sequence
FESEM	Field Emission Scanning Electron Microscopy
FESEM-EDS	Field Emission Scanning Electron Microscopy-Energy Dispersive Spectroscopy
PV	Pore Volume
θ	Theta
KE	Kinetic energy of rain per unit precipitation

E	Kinetic energy of rainfall
ASTM	American Society for Testing and Materials
IS	Indian standard code
$\mathbf{T_p}$	Time to ponding
VWC	Volumetric water content