Kochi Chapter

Indian Geotechnical Conference
IGC 2022
15th – 17th December, 2022, Kochi

Effect of Microbial Enzyme on Fly ash and Assessment of Compressive Strength

Joyprakash Naskar[1][0000-0002-9384-9497] and Anil Kumar Sharma[1][0000-0001-9809-4700]

[1] Department of Civil Engineering, National Institute of Technology Patna, Patna-800005

aks.ce@nitp.ac.in

Abstract. Microbial induced calcite precipitation is an emerging technique in the field of soil stabilization techniques. In the present on-going study, the stabilization of fly ash is investigated through the MICP process. *Sporosarcina pasteurii*, a natural urease enzyme producing bacteria, has been considered because previous studies demonstrated that *Sporosarcina pasteurii* has a high urease enzyme producing capacity and can hydrolyze more than 90% of urea in a comparatively shorter time span. A urease enzyme is a type of enzyme that hydrolyzes urea and precipitates it as calcium carbonate ($CaCO_3$). Two different cell concentrations of these microbes at $OD600_{nm}$ (0.8) along with a binding solution of urea and calcium chloride passed through a fly ash column of length and diameter (2:1) ratio. After microbial treatment, fly ash samples were subjected to an Unconfined Compressive Strength (UCS) test. The test results show improvement in UCS value with treatment. The precipitation of calcium carbonate is the key material which binds with fly ash particles. The goal of this study is to look at fly ash as a building material and see if it can be used in the field.

Keywords: MICP; Fly ash; *Sporosarcina Pasteuri*; Unconfined Compressive Strength; Sustainability.

1 Introduction

Sustainable building material demand is increasing day by day. Some traditional construction materials like sand, cement, and aggregates have been used in previous decades. Today, the number of thermal power plants is increasing day by day to meet the demand for power requirements. Hence, fly ash generation is also high. Recycling and reuse of fly ash is an economical and eco-friendly technique to conserve natural resources for sustainable development. The use of fly ash as a building material is recognized worldwide [1] To prevent the adverse environmental impact of fly ash, recycling rates are increasing to minimize its negative impact and promote better sustainable development [2- 3].

In recent years, bio-cementation, which is based on calcite precipitation using microbes, has been recognized worldwide as a novel consolidation technique, used in cement concrete mortars, expansive soil and sand consolidation to improve engineering properties and compressive strength [4-6]. A novel method that uses urease-producing microbes to produce carbonate has been introduced and examined by prior

studies to address the remediation of metal leaching from CFA. Microbially induced carbonate precipitation (MICP) is a well-known name for this method [7-10]. By using MICP to immobilize cadmium in soils, Kumari et al. (2014) observed a 90% conversion of soluble cadmium to carbonate-bound cadmium [11]. Microbe induced calcium carbonate precipitation is a mineralization process leading to $CaCO_3$ precipitation to fill the pore structure of fly ash and bind them. The microbe reaction process induces calcite precipitation via urea hydrolysis by urease enzyme as follows:

- Urea gets hydrolyzed by the urease enzyme and produces carbamate and ammonia:
  $CO(NH_2)_2 + H_2O \longrightarrow NH_2COOH + NH_3$ (i)
- After hydrolysis of carbamate, 1 mole of ammonia and carbonic acid are formed:
  $NH_2COOH + H_2O \longrightarrow NH_3 + H_2CO_3$ (ii)
- Carbonic acid dissociates into one mole of bicarbonate and a hydrogen ion:
  $H_2CO_3 \longrightarrow H^+ + HCO3^-$ (iii)
- Ammonia dissociates into 2 moles of ammonium ions and hydroxide ions:
  $2NH_3 + 2H_2O \longrightarrow 2NH_4^+ + 2OH^-$ (iv)
- Due to the presence of ammonium ion, pH increases and bicarbonate ions dissociate and carbonate ions are formed and a combined reaction occurs:
  $HCO_3+ + H^+ + 2NH_4^+ + 2OH^- \longrightarrow CO_3^{2-} + 2NH_4^+ + 2H_2O$ (v)
- Bacterial cell walls are negatively charged. Due to this, bacterial cell walls attract $Ca2^+$ and cations and stick to the cell surface; further $Ca2^+$ reacts with $CaCO_3$ and precipitation of calcium carbonate occurs at the bacterial cell surface as a nucleation site and the size of CaCO3 mineral increases:
  $Cell + Ca^{2+} \longrightarrow (Cell\text{-}Ca^{2+})$ (vi)
  $(Cell\text{-}Ca^{2+}) + CO_3^{2-} \longrightarrow (Cell\text{-}CaCO_3)$ (vii)

This study focuses on improving the compressive strength of fly ash by using bacterial solutions and cementation solutions. Nutrients, calcium, and urea have been given as ingredients for bacterial growth and bio cementation.

2 Materials

2.1 Source of bacteria

Bacteria, namely *Sporosarcina pasteurii* (MTCC 1761), were procured from the Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh, India.

2.2 Nutrients for bacterial growth

LB (Luria-Bertani) Broth was used as a nutrient for bacterial species as shown in Table 1.

Table 1. Nutrient Source

Ingredients	Gram/Litre
Tryptone	10.00
Yeast extract	5.00
Sodium chloride	10.00

The nutrients were mixed with distilled water and autoclaved for 15 minutes at 121^{o} C.

2.3 Biocementation medium preparation

Table 2. Biocementation composition

Mineral	Molarity
Urea	0.5
Calcium chloride	0.5

The biocementation solution was prepared and pH was adjusted to 7.5 with 1 N sodium hydroxide as shown in Table 2.

2.4 Source of fly ash

A fly ash sample was collected from the National Thermal Power Corporation located at Barh in Bihar, India. Samples were transported with the utmost precaution for studying various properties of fly ash samples.

3 Methodology

3.1 Microbial solution preparation

Frozen *Sporosarcina pasteurii* was inoculated in LB (Luria Bertani) broth nutrient medium and placed in an incubator at 37^{o}C for 24 hours. The bacterial culture was then sub-cultured in 250 ml of LB broth. Biological solution was checked at OD (Optical Density) at 600_{nm} wave length for determining cell concentration. In this study, an $OD600_{nm}$ of 0.8 was used and cell concentration was taken as 6.34107 cells/ml. The bacterial cell concentration in growth media was measured by the expression [12].

$$Y = 8.59 \times 10^{7} \times Z^{1.3627} \quad \text{(viii)}$$

Note: ($Z = OD_{600nm}$ reading, Y = cell concentration per milliliter)

3.2 Cementation solution preparation

Urea and calcium chloride were mixed in distilled water used as a cementation media preparation. Table 2 shows the detailed composition of cementation media. In addition, 3gm of LB broth per litre was added for the supply of nutrients to bacteria [13]. Nutrients are added for the multiplication of bacteria and a higher ureolysis rate.

3.3 Physical properties of fly ash

On the collected fly ash sample, various tests such as specific gravity, gradation, and standard proctor test were performed in accordance with the IS-2720 [14-19]. Unconfined compressive strength was conducted on an untreated fly ash sample and a MICP-treated fly ash sample. Table 3 gives different physical and engineering properties of a fly ash sample.

Table 3. Physical and engineering properties of fly ash

Properties	Value
Specific gravity (G)	2.01
% Finer than 75 μm	40.04
Sand (%)	59.96
Silt (%)	40.04
Coefficient of Uniformity (Cu)	7.86
Coefficient of Curvature (Cc)	1.21
Optimum Moisture Content (%)	31.47
Maximum Dry Unit Weight (kN/m3)	11.22
Unconfined compressive strength, untreated(kPa)	---
Unconfined compressive strength, MICP treated(kPa)	422
pH	7.85

3.4 Specimen preparation

A fly ash column was prepared in a cylindrical PVC mould of 40 mm in diameter and 90 mm in length to maintain a ratio of greater than 2 (height: diameter). Moulds were prefabricated and formed at the National Institute of Technology Patna as shown in Fig. 1. Twelve numbers of oven dried fly columns were prepared by adding the bacterial solution at OMC. PVC moulds were fitted with silicon pipe at both ends and made ready for treatment.

$$(NH_2)_2CO + 2H_2O \rightarrow CO_2 + H_2O + NH_3 \quad \text{(ix)}$$

Fig. 1. PVC moulds for specimen preparation

3.5 MICP treatment process

Fig. 2 shows the adopted method of MICP treatment in this study. A fly ash column was placed in a vertical position with clamps and stands. One end of a silicon pipe was fitted with a peristaltic pump to maintain a constant rate of flow of bacterial media and cementation solution. The other end of the silicon pipe is placed in a container to collect effluent. At the time of column preparation at OMC, two pore volumes of cementation solution were passed through the fly ash column and retained for 10 hours. After that, one pore volume of bacteria medium was given to the sample and retained for two hours. After that, two pore volumes of cementation solution were given to the fly ash sample again. This cycle continues for 5 days, after which the samples are cured for seven days. Thereafter, samples were dried as prepared for the unconfined compressive strength test.

Fig. 2. MICP treatment process through peristaltic pump

4 Result and Discussion

4.1 Urease test

The hydrolysis of bacteria is confirmed by a urease test. In this process, after hydrolysis, urea produces ammonia and carbon dioxide. Ammonia increases the basicity of the medium. Phenol red reagent is used as an indicator. It changes colour from light orange (pH 6.8) to magenta (pH 8.1). Urease enzyme producing bacteria turn the medium pink within 24 hours [20]. 10 ml of cementation solution and 5 ml of bacterial solution were mixed in a test tube and placed in an incubator at 37^{o}C for 24 hours. Colour change is detected and it confirms that it is a urease enzyme producing bacteria and it is efficient for hydrolysis of urea. Figs. 3 and 4 show the confirmation of urease activity of *Sporosarcina pasteurii*.

$$(NH_2)_2CO + 2H_2O \rightarrow CO_2 + H_2O + NH_3 \quad \text{(ix)}$$

Fig. 3. Urease activity test at time T_0

Fig. 4. Urease activity test result after 24 hours.

4.2 Compressive strength test

Unconfined compressive strength (UCS) [21] tests were carried out to determine the compressive strength of untreated and treated cylindrical samples of fly ash. However, it must be highlighted that the UCS value of untreated samples was very negligible. In a similar study, bio treated fly ash samples developed strength of about 33.6 kPa [22]. As shear strength becomes a governing design criterion for geotechnical engineering constructions, numerous studies on the application of MICP to improve the shear strength of soil and related materials have been done [23,24]. Almost all of these studies revealed an increase in UCS ranging from 36 to 12,400 kPa. The stress-strain behavior of fly ash samples treated with MICP for seven days is depicted in Fig. 5. Similar improvement in UCS values has been observed by other researchers. In case of fine sand, the UCS values of samples stimulated for both aerobic and anaerobic bacteria growth increased by 254–283% [25]. In an experimental investigation of the MICP treatment of calcareous foundations on an artificially reclaimed island, the UCS reached around 821 kPa after nine MICP treatments [26]. The treated fly ash samples attained a compressive strength of 422 kPa. As observed, it shows a significant increase in strength in comparison to untreated samples, for which the UCS value was negligible. Fig. 6 shows the tested samples after the UCS test. Therefore, it may be concluded that MICP treatment is also effective in stabilizing fly ash.

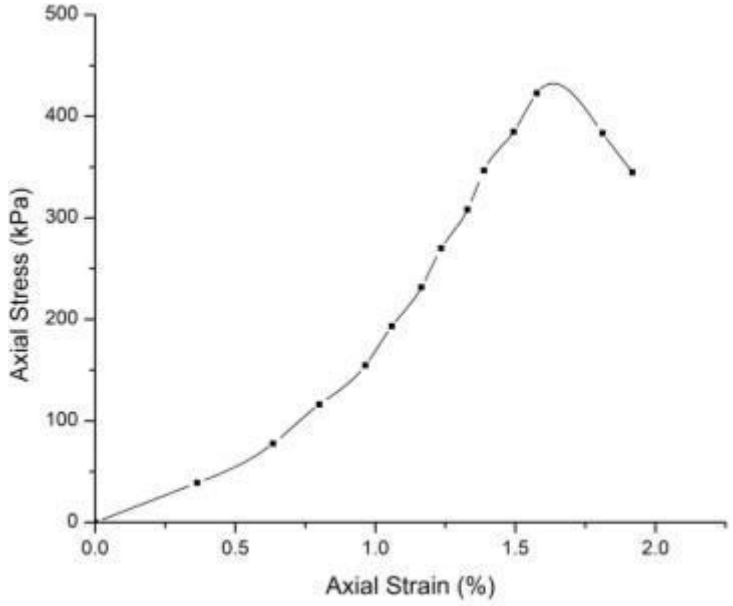

Fig. 5. Stress-strain curve for MICP treated fly ash after 7 days

Fig. 6. MICP treated fly ash sample after UCS test

5 Conclusion

Based on this experimental study, it can be concluded that Microbial induced calcite precipitation (MICP) has been found to be effective in improving the UCS of fly ash. The unconfined compressive strength of fly ash increased after 7 days of MICP treatment and the UCS value is 422 kPa. That is quite impressive at OD600 of 0.8 bacterial concentrations and 0.5 M of biocementation solution concentration. Further investigation is need to establish the effect of MICP with other engineering properties such as compressibility and permeability characteristics.

References

1. Su, P., Zhang, J., & Li, Y. Solidification/stabilization of stainless steel pickling residue with aluminum potassium sulfate amended fly ash. Journal of Cleaner Production, 234, 400-409. (2019)
2. Bhattacharyya, P., Reddy, K. J., & Attili, V. Solubility and fractionation of different metals in fly ash of Powder River Basin Coal. Water, Air, & Soil Pollution, 220(1), 327-337. (2011)
3. Cetin, B., Aydilek, A. H., & Li, L. Experimental and numerical analysis of metal leaching from fly ash-amended highway bases. Waste management, 32(5), 965-978. (2012)
4. Seifan, M., Samani, A. K., & Berenjian, A. Induced calcium carbonate precipitation using Bacillus species. Applied microbiology and biotechnology, 100(23), 9895-9906. (2016)
5. Xu, H., Zheng, H., Wang, J. N., Ding, X. Q., & Chen, P. Laboratory method of microbial induced solidification/stabilization for municipal solid waste incineration fly ash. MethodsX, 6, 1036-1043. (2019)

6. Li, M., Fang, C., Kawasaki, S., & Achal, V. Fly ash incorporated with biocement to improve strength of expansive soil. Scientific reports, 8(1), 1-7. (2018)
7. Zhang, J., Wen, K., & Li, L. Bio-modification of coal fly ash using urease-producing bacteria. Fuel, 286, 119386. (2021)
8. Chen, P., Zheng, H., Xu, H., Gao, Y. X., Ding, X. Q., & Ma, M. L. Microbial induced solidification and stabilization of municipal solid waste incineration fly ash with high alkalinity and heavy metal toxicity. PloS one, 14(10), e0223900. (2019)
9. Xu, H., Zheng, H., Wang, J. N., Ding, X. Q., & Chen, P. Laboratory method of microbial induced solidification/stabilization for municipal solid waste incineration fly ash. MethodsX, 6, 1036-1043. (2019)
10. Kadapure, S. A., Kulkarni, G. S., & Prakash, K. B. A laboratory investigation on the production of sustainable bacteria-blended fly ash concrete. Arabian Journal for Science and Engineering, 42(3), 1039-1048. (2017)
11. Kumari, D., Pan, X., Lee, D. J., & Achal, V. Immobilization of cadmium in soil by microbially induced carbonate precipitation with Exiguobacterium undae at low temperature. International Biodeterioration & Biodegradation, 94, 98-102. (2014)
12. Ramachandran, S. K., Ramakrishnan, V., & Bang, S. S. Remediation of concrete using micro-organisms. ACI Materials Journal-American Concrete Institute, 98(1), 3-9. (2001)
13. Cheng, L., Shahin, M. A., Cord-Ruwisch, R., Addis, M., Hartanto, T., & Elms, C.. Soil stabilisation by microbial-induced calcite precipitation (MICP): investigation into some physical and environmental aspects. In 7th international congress on environmental geotechnics (Vol. 64, No. 12, pp. 1105-1112). Engineers Australia Melbourne, Australia. (2014)
14. IS : 1498 – 1970, Classification And Identification of Soils for General Engineering Purposes, (First Rev.), Bureau of Indian Standard (BIS).
15. IS : 2720 (Part 1) – 1983, Preparation of Dry Soil Samples for Various Tests, (Second Rev.), Bureau of Indian Standard (BIS).
16. IS : 1498 – 1970, Classification and Identification of Soils for General Engineering Purposes, (First Rev.), Bureau of Indian Standard (BIS).
17. IS : 2720 (Part VII) -1980, Determination of Water Content-Dry Density Relation Using Light Compaction, (Second Rev.), Bureau of Indian Standard (BIS).
18. IS : 2720 (Part 13) - 1986, Direct Shear Test, (Second Rev.), Bureau of Indian Standard (BIS).
19. IS : 2720 (Part III/Sec 1) - 1980, Determination of Specific Gravity, (First Rev.), Bureau of Indian Standard (BIS).
20. Morgan, A., Babu, D., Reiz, B., Whittal, R., Suh, L. Y., & Siraki, A. G. Caution for the routine use of phenol red–it is more than just a pH indicator. Chemico-Biological Interactions, 310, 108739. (2019)
21. IS 2720 (Part 10) : 1991, Determination of Unconfined Compressive Strength, Bureau of Indian Standard (BIS).
22. Yadav, A., Vineeth Reddy, K., Khan, M., Kalyan Kumar, G., & Bandhu, A.. Bio-treatment of Fly ash. In Advances in Computer Methods and Geomechanics (pp. 505-517). Springer, Singapore. (2020)
23. Fatehi, H., Abtahi, S. M., Hashemolhosseini, H., & Hejazi, S. M. A novel study on using protein based biopolymers in soil strengthening. Construction and Building Materials, 167, 813-821. (2018)
24. Chen, R., Ramey, D., Weiland, E., Lee, I., & Zhang, L. Experimental investigation on biopolymer strengthening of mine tailings. Journal of Geotechnical and Geoenvironmental Engineering, 142(12), 06016017. (2016)
25. Pakbaz, M. S., Kolahi, A., & Ghezelbash, G. Assessment of Microbial Induced Calcite Precipitation (MICP) in Fine Sand Using Native Microbes under Both Aerobic and Anaerobic Conditions. KSCE Journal of Civil Engineering, 26(3), 1051-1065. (2022)
26. Liu, H. L., Ma, G. L., Xiao, Y., Ding, X. M., & Fang, X. W. In situ experimental research on calcareous foundation stabilization using MICP technique on the reclaimed coral reef islands. Chinese Ground Improvement, 1(1), 26-31. (2019)