Calcite mineral characterization and quantification of urease enzyme mediated sand columns

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Abstract. In this paper urease enzyme facilitated calcium carbonate precipitation for soil strength improvement is characterized using X-ray diffraction and the amount of calcium carbonate precipitated is quantified based on acid leaching, thermal decomposition and CO2 volume evaluation approaches. These techniques are adopted to evaluate the spreading of calcium carbonate crystals within the sand matrix after treatment with urease enzyme and nutrients. Sand columns of 60mm diameter and height 60 mm are prepared with the help of PVC pipes. Nutrient solution made up of urea, urease enzyme producing Sporosarcina pasteurii bacterium and calcium supplement in the form of calcium chloride is introduced into the sand columns ensuring the uniform distribution. Later treated samples of sand is examined for calcium carbonate precipitation by Sporosarcina pasteurii bacterium by XRD and quantified.

Keywords: Urease enzyme; soil-stabilization, Sporosarcina pasteurii, Thermal decomposition, CO2 volume evaluation

1. Introduction
Urease enzyme facilitated calcium carbonate precipitation technique (UEFCCP) has found many applications such as detoxification of ground water from contaminants, crack remediation in concrete, restoration and preservation of limestone structures, controlling the swelling nature of clay soils, to stabilize soil from liquefaction during earthquakes etc. In UEFCCP technique, the calcium carbonate is precipitated due to reversible microbiological reactions in which the urea is hydrolyzed by urease enzyme into ammonia and carbonate ions. Ammonia will maintain the alkalinity of the green concrete and carbonate ions combine with calcium ions from supplied calcium nutrient to form calcite minerals. Due to its significant cementing capability this technique have been used for many civil engineering applications such as healing the cracks in concrete, improving the mechanical properties of the soil by binding its
particles. The major challenge for this UEFCCP technique is to extend for in-situ application scale from the laboratory scale because UEFCCP technique needs sterilized approach for its bacterial activity. Few issue of this technique are (1) application to soils is that in case of fine grained soil the movement of bacteria is restricted (2) if the nutrients solution is used more than required then it may affect the bacterial activity (3) use of bacteria in soil with nutrients has problems with enzyme fixation and culturing.

So the precipitation of calcite crystals can be done using microbial method or by enzymatic method. In first method bacteria and nutrients are introduced into the soil where as in enzymatic method enzyme is injected along with the reagent. Calcite mineral diffusion in soil sample plays a major role so means to disperse urease enzyme produced calcite crystals are to be paid special attention. Unconfined compression tests will be conducted to assess the strength of the soil sample.

Nutrients broth consisting of urea and calcium supplement is used to culture different concentrations of Sporosarcina pasteurii which produces enzyme that decomposes urea. The one optimal concentration of Sporosarcina pasteurii will be added to PVC column soil samples. The soil getting stiff is observed.

2. Calcite quantification methods

2.1 Acid Leaching Method (ALM)

The precipitated calcite mineral can be quantified using this acid leaching method in which oven dried out bacteria treated soil samples are washed with HCL acid numerous times so that calcite mineral present in the soil sample will be washed out. Later the soil sample is washed with water and oven dried. So the loss of mass of acid washed and oven dried bacteria treated soil sample gives the amount of calcite mineral present in the soil sample. This method may overestimate the amount of calcite mineral precipitation because when the bacteria treated soil sample is washed with acid and water for at least 3 to 5 times during which not only calcite mineral is washed away but the soluble materials present in the soil sample may also get removed resulting in little more loss of mass.

Also this method is little time consuming because it has to be washed and oven dried many times. So to quantify the bacterially generated calcite crystals more accurately the thermal decomposition and CO2 volume evaluation approaches are experimented to evaluate the reliability and speed with which these methods can quantify calcite crystals. Past researchers assessed the calcite precipitation even by measuring the S-wave velocity.

Three similar soil samples are chosen and treated with bacteria and are quantified for calcite mineral precipitation using three approaches such as acid leaching, thermal decomposition and CO2 volume evaluation methods. Commercially available calcium carbonate of 98% purity is also obtained for calibration purpose.
Table 1. Results of Acid leaching method

| Sample Type | Mass of Sand (gm) | Mass of CaCO₃ (gm) | Total Mass (gm) | Total Mass after acid and water wash (gm) | Loss of mass (gm) | Percentage Loss |
|-------------|-------------------|--------------------|-----------------|------------------------------------------|------------------|----------------|
| 1           | 50.0              | 0.0                | 50.0            | 49.936                                   | 0.064            | 0.13           |
| 2           | 49.5              | 0.5                | 50.0            | 49.748                                   | 0.252            | 0.50           |
| 3           | 49.0              | 1.0                | 50.0            | 49.648                                   | 0.352            | 0.70           |
| 4           | 48.5              | 1.5                | 50.0            | 49.464                                   | 0.536            | 1.07           |
| 5           | 48.0              | 2.0                | 50.0            | 49.292                                   | 0.708            | 1.42           |
| 6           | 47.5              | 2.5                | 50.0            | 49.124                                   | 0.876            | 1.75           |
| 7           | 47.0              | 3.0                | 50.0            | 48.852                                   | 1.148            | 2.30           |
| 8           | 46.5              | 3.5                | 50.0            | 48.756                                   | 1.244            | 2.49           |
| 9           | 46.0              | 4.0                | 50.0            | 48.444                                   | 1.556            | 3.11           |
| 10          | 45.5              | 4.5                | 50.0            | 48.356                                   | 1.644            | 3.29           |
| 11          | 45.0              | 5.0                | 50.0            | 48.264                                   | 1.736            | 3.47           |

Figure 1. Calibration curve plotted between loss of mass after heating and mass of calcium carbonate mixed used for Acid leaching method to quantify calcite.
2.2 Thermal decomposition method (TDM)

This method is based on the principle that the calcium carbonate mineral will decompose at temperature of around 591°C into calcium oxide and carbon dioxide and may lose nearly 45% of its mass.

Table 2. Results of thermal decomposition method

| Sample Type | Mass of Sand (gm) | Mass of CaCO₃ (gm) | Total Mass of mixture (gm) | Total Mass after thermal exposure (gm) | Loss of mass (gm) | Percentage Loss |
|-------------|------------------|--------------------|--------------------------|----------------------------------------|------------------|-----------------|
| 1           | 50.0             | 0.0                | 50.0                     | 49.920                                 | 0.080            | 0.16            |
| 2           | 49.5             | 0.5                | 50.0                     | 49.685                                 | 0.315            | 0.63            |
| 3           | 49.0             | 1.0                | 50.0                     | 49.560                                 | 0.440            | 0.88            |
| 4           | 48.5             | 1.5                | 50.0                     | 49.330                                 | 0.670            | 1.34            |
| 5           | 48.0             | 2.0                | 50.0                     | 49.115                                 | 0.885            | 1.77            |
| 6           | 47.5             | 2.5                | 50.0                     | 48.905                                 | 1.095            | 2.19            |
| 7           | 47.0             | 3.0                | 50.0                     | 48.565                                 | 1.435            | 2.87            |
| 8           | 46.5             | 3.5                | 50.0                     | 48.445                                 | 1.555            | 3.11            |
| 9           | 46.0             | 4.0                | 50.0                     | 48.055                                 | 1.945            | 3.89            |
| 10          | 45.5             | 4.5                | 50.0                     | 47.945                                 | 2.055            | 4.11            |
| 11          | 45.0             | 5.0                | 50.0                     | 47.830                                 | 2.170            | 4.34            |

The table 2 shows various mixture combinations of over-dried sandy soil and commercial calcium carbonate powder. A known composition of calcium carbonate and sand are mixed to make mixture of total mass 50 gm and placed in muffle furnace. After exposed to 600 °C temperature for 6 hrs., the loss of mass is computed which will indirectly give the mass of carbon dioxide lost as gas. So for various compositions of calcium carbonate and oven-dried sand samples, loss of masses of mixture after heating for 6 hrs. at 600 °C are recorded and a calibration curve is plotted between loss of mass after heating and mass of calcium carbonate mixed. Due to high temperature there may be loss in mass of sand also due to decomposition of impurities. It was found that 0.080 gm of loss of mass was observed when the sand is heated to stipulated temperature. This error can be incorporated by making suitable corrections in the loss of masses of other mixtures.
Figure 2. Calibration curve plotted between loss of mass after heating and mass of calcium carbonate mixed used for thermal decomposition method to quantify calcite.

The calibration curve plotted between loss of mass after heating and its corresponding mass of calcium carbonate mixed used can be used to quantify calcite using thermal decomposition method. It is apparent that the mass loss in the thermal decomposition method is more than the acid leaching method due to decomposition of other minerals or impurities present in the sand sample.

2.3 CO₂ volume evaluation method (CVEM)

The principle behind this method is that when HCL acid is added to the calcium carbonate, carbon dioxide gas will be evolved along with calcium salt. During the neutralization of calcium carbonate, carbon dioxide gas evolved is directly correlated to the quantity of calcium carbonate disintegrated. So if all the calcite minerals are expended then the gas collected and measured will quantify the amount of calcite precipitated.
Figure 3. Carbon dioxide gas volume measurement test setup

This method was suggested to be most reliable as far as calcite quantification is concerned. A 50 grams of calcium carbonate mixed sand is taken into the flask and neutralized with the 50 mL HCL of 2.5 mol/L. It was estimated that the 50 mL HCl of 2.5 mol/L will decompose 12.5 gm of calcium carbonate. Shake the flask so that calcium carbonated sand and HCL to mix thoroughly so that gas bubbles of carbon dioxide will evolve. The evolved CO$_2$ gas passes through the tube and moves the water down in the measuring jar. So the amount of HCL acid added will produce gas which will displace equal amount of water in the measuring jar. Even in sand with no calcium carbonate also some amount of water is displaced means that some impurities also reacts and releases gas so this amount of correction is applied in subsequent measurements of gas from sand with calcium carbonate. The table XXX shows the amount of gas released for corresponding quantity of calcium carbonate added to sand.

Table 3. Amount of gas released after reacting with HCL

| Sample Type | Mass of Sand (gm) | Mass of CaCO$_3$ (gm) | Total Mass of mixture (gm) | Volume of gas cm$^3$ |
|-------------|------------------|------------------------|---------------------------|----------------------|
| 1           | 50.0             | 0.0                    | 50.0                      | 50                   |
| 2           | 49.5             | 0.5                    | 50.0                      | 115                  |
| 3           | 49.0             | 1.0                    | 50.0                      | 225                  |
| 4           | 48.5             | 1.5                    | 50.0                      | 360                  |
| 5           | 48.0             | 2.0                    | 50.0                      | 460                  |
| 6           | 47.5             | 2.5                    | 50.0                      | 570                  |
| 7           | 47.0             | 3.0                    | 50.0                      | 670                  |
| 8           | 46.5             | 3.5                    | 50.0                      | 755                  |
| 9           | 46.0             | 4.0                    | 50.0                      | 850                  |
| 10          | 45.5             | 4.5                    | 50.0                      | 945                  |
| 11          | 45.0             | 5.0                    | 50.0                      | 1025                 |
When the bacteria treated sand samples were quantified for calcite using acid leaching method, thermal decomposition method and carbon dioxide volume evaluation method, it was observed that the calcite quantified using these methods are varying considerably. Amount quantified by acid leaching method is more due to presence of flushed soluble impurities other than calcite mineral. Similar observation is made in thermal decomposition method where ammonium chloride which is formed along with the calcium chloride evaporates at 600 °C temperature. Ammonium chloride formed with calcite mineral precipitation is inert in HCL in case of acid leaching method but it is soluble so it has no effect on the strength properties.

3. Conclusion
Based on three methods of quantifying the precipitated calcite mineral in sand samples. The following conclusions are established-

1. Urease enzyme facilitated calcium carbonate precipitation, has been appraised for its likely use in improving the soil properties.
2. Three calcite quantification methods that employ the elementary philosophies of chemistry have been assessed.
3. The tests conducted show that the Measuring volume of CO\textsubscript{2} method seems to be appropriate for measuring the precipitated calcite mineral.

4. References

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