CALCIUM CARBONATE PRECIPITATING YEAST ON CEMENT CONCRETE

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Abstract

Bio-mediated soil improvement is an interdisciplinary field collaborated with microbiology, geochemistry and civil engineering for ground improvement. This process is technically termed as microbially induced calcite precipitation. Naturally most of the microbes involved in mineral precipitation especially carbonates. This is the work on calcium carbonate precipitation by yeast and applied on cement concrete to observe the strength enhancement point of view. Like ureolytic bacteria, isolated ureolytic soil yeast would involve urea hydrolysis and favours to calcium carbonate precipitation. Both growth and precipitation conditions were studied and strength of precipitated concrete was observed.

Key words: Yeast; concrete; mineralization; calcium carbonate; Urease

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Introduction

Microbially induced calcite precipitation is a chemical process in which the microbe consumes and breaks down urea to form ammonia, bicarbonate and carbonate ions. The calcium ions within the medium fed to the microbes which are then free to bond with the carbonate to form a level of cementation on each sand grain. This makes a more cohesive bond within the soil sample particle matrix as it is one of the most reactive and common minerals found in the earth's surface. During this process, the ammonia plays an important role as it helps increase the pH making an ideal environment for the bacteria to feed on the urea and precipitate calcite.

Unicellular fungi are called yeasts. Soil yeasts contribute to essential ecological processes such as the mineralization of organic material and dissipation of carbon and energy through the soil ecosystem. Yeasts are ubiquitous in their distribution and populations mainly depend on the type and concentration of organic materials. The distribution of species as well as their numbers and metabolic characteristics were found to be governed by existing environmental conditions (Botha,2011)

Calcium carbonate precipitation is a straight forward chemical process by four key factors: the calcium concentration; Dissolved inorganic carbon concentration; pH and the availability of nucleation sites. The precipitation pathways are generally found in nature which accounts for the common occurrence of microbial carbonate precipitation and accordance to the statement, that under suitable conditions, most of the microbes having the capacity of inducing carbonate precipitation. The most common system of applied microbially induced calcium carbonate is urea hydrolysis by the enzyme urease in a calcium rich environment. Urease catalyses urea converted to CO2 and ammonia, resulting in an increase of pH and carbonate concentration in micro-environment. During urease activity, 1 mol. of urea is hydrolysed to 1 mol of ammonia and 1 mol of carbonate, which spontaneously hydrolyzes to form additional 1 mol of ammonia and carbonic acid.

$$CO(NH_2)_2 + H_2O \rightarrow NH_2COOH + NH_3$$

$$NH_2COOH + H_2O \rightarrow NH_3 + H_2CO_3$$

These products equilibrate in water to form bicarbonate, 1 mol. of ammonium and hydroxide ions which give rise to pH increase

$H_2CO_3 \longrightarrow 2H^+ + 2CO_3^{2-}$

$NH_3 + H_2O \longrightarrow NH_4^- + OH^-$

$Ca^2 + CO_3^{2-} \longrightarrow CaCO_3$

$Ca^{+2} + cell \longrightarrow cell\text{-}Ca^{+2}$

$Cell\text{-}Ca^{+2} + CO_3^{-2} \longrightarrow cell\text{-}CaCO_3$

The above series of events occurring during ureolytic calcification proved the importance of pH, possible biochemical reactions in urea-$CaCl_2$ medium to precipitate $CaCO_3$ at the cell surface (Casteiner et al, 2000; Mayur and Pitroda, 2013).

During the microbially induced carbonate precipitation, Calcium ions in the solution are attracted to the cell wall due to the negative charge of the latter. Upon addition of urea, dissolved inorganic carbon and ammonium are released in the microenvironment.

Urease is a nickel-containing enzyme that catalyses the hydrolysis of urea into ammonia and carbon dioxide. This enzyme occurs in such different organisms as bacteria, algae, yeast like fungi, fungi and higher plants. Its primary function is allowing the organism to use urea as a nitrogen source (Fidaleo and Lavecchia, 2003).

Materials and methods

Isolation of *C.tropicalis*

One gram of collected soil from agricultural field was serially diluted with distilled water and plated on Sabourd dextrose agar medium. 48 hours matured cells were used for further studies.

Gram staining

Heat fixed prepared smear on a glass slide was flooded with crystal violet for a minute and then washed gently in water to remove excess. Then it was flooded with Gram's iodine, then washed with water and then flooded by counter stain saffranin for 15 sec and washed with water to remove excessive stain. Strain was visualized under microscope.

Growth was red spectrophotometrically at 600nm.

Urease test

Isolate was tested for urease activity, which was done by tryptic soy broth culture containing urea (20g/l), Na2HPO4 (9.5g/l), KH2PO4 (9.1g/l), Yeast extract (0.1g/l) and 0.01g phenol. pH was made to 6.8+/- 2. This test detects the ability of organism to produce urease enzyme. This enzyme converts urea to ammonia and co2.

Calcium carbonate precipitating medium

The calcium carbonate producing compositions are: urea 20g/l; NaHCO3 2.21g/l; NH4Cl 10g/l; CaCl2.2H2O 25g/l; SD broth 3g/l.

Concentration of Dissolved calcium ions

Calcium ion concentration was measured by ethylene diamine tetracidic acid (EDTA) titration. DisodiumEDTA is commonly used to standardize aqueous solutions of transition metal cations. DisodiumEDTA only forms four coordinate covalent bonds to metal cations at pH values ≤12. The main reason that EDTA is used so extensively in the standardization of metal ions solutions is that the formation constant for most metal cation-EDTA complexes is very high, measuring that the equilibrium for the reaction. The completion of the reaction detects from the formation of the metal cation-EDTA complex and it is chiefly used in titrations or standardization. Eriochrome Black T is used as a complexometric indicator to determine the end point. EBT is an organic dye which displaces the metal cation and reached the end point with as metallic blue. So the free indicator is serves as the end point indicator.

Decreasing calcium ions favours calcium carbonate precipitation increase.

Urease activity

According to the procedure from Natarajan et al., 1995, Urease activity was calculated. From the standard graph (R2=0.991) of Ammonium chloride (100µg/ml)

Preparation of cement concrete

M15 grade (1:2:4 = cement: gravel: sand) concrete cubes were prepared about 15×15×15Cm size.

During curing, the cubes were stored in moist air for 24 hours and after removed from the molds kept submerged in clear fresh water until for test. The temperature curing water was 27±2°C.

Compressive strength = (Load in N/ Area in $mm^{2)}$

Results

Isolated soil yeast as *C.tropicalis*

On Sabourd Dextrose agar - Creamy white colour with mycelia border
Gram staining - Spherical budding yeast
Germ tube test – Negative
On CHROMagar - Bluish purple colour (Ainscough and Kibbler, 1998)
Indian ink preparation – No capsule
Urea hydrolysis – Positive
Calcium carbonate production - 1.48g/100ml
Urease activity - 5.2u/ml
Optimum growth conditions - 35°C and 8 pH
Optimum precipitation - 35°C and 8.5 pH

28days cured cement concrete was immersed in equimolar solution of urea-calcium chloride containing SD medium for 48 hours. Precipitation was occurred on the surface of concrete which reduce the pores enhance strength of cement concrete. 28 days cured concrete was considered as control specimen and the equimolar solution treated concrete was considered as test specimen. Both the control and test specimens were involved for compressive strength was calculated in percentage. Percentage of strength in control specimen was 96% and the test specimen shows 99%. Ureolytic yeast precipitated calcium carbonate on the surface can enhance concrete strength.

Conclusion

This work concluded that the isolated soil yeast *Candida tropicalis* can hydrolyse urea and enhance the biochemical reaction for precipitating calcium carbonate. At pH 8.5 and 35C, optimal calcium carbonate precipitation was observed. Also proved that the precipitation on cement concrete enhance the compressive strength.

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