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Effect of different carbon sources in biogenic calcium carbonate production by Bacillus subtilis 168.

Hector Ferral Perez [1], Monica Galicia García [1], Marisela Aguirre Ramírez [1], Aldo Izaguirre Pompa [2], Bonifacio Alvarado Tenorio [1]

[1] *Departamento de Ciencias Químico-Biológicas, Universidad Autónoma de Ciudad Juárez, Instituto de Ciencias Biomédicas, Cd. Juárez, Chih., 32310. México.*

[2] *Departamento de Ingeniería Civil y Ambiental, Universidad Autónoma de Ciudad Juarez, Instituto de Ingeniería y Tecnología, Cd. Juárez, Chih.,32310, México.*

Calcium carbonate ($CaCO_3$) is one of the most widely inorganic compounds used in several industries, such as plastics, cosmetics, pharmaceutic, construction and paper. Also, is one of the most abundant mineral in nature, being part of several geocycles, both in marine and lake sediments [1]. $CaCO_3$ mineralization by bacterial metabolism is a promising biotechnological environmental friendly technique to develop new biomaterials. Bacterial $CaCO_3$ mineralization is a phenomenon that occurs in sediments, in caves and even in monuments and buildings [2], [3], [4]. The ability of bacteria to nucleate calcium ions and form minerals is due to the production and nature of exopolymeric substances (EPS) and biofilm geometry [5]. Several investigations have reported differences in mineralizing yield, crystallinity and polymorphic products in different growth condition and species dependence (e.g., strains of *Bacillus licheniformis* and *Lysinubacillus sphaericus* (*B. sphaericus*)) [6]. The purpose of this work is to determine the conditions of $CaCO_3$ crystals production by *Bacillus subtilis* in a semi-solid media supplemented with different carbon and calcium sources.

$CaCO_3$ crystals formation was performed in semisolid media supplement with different carbon source, 2 calcium linked, (calcium acetate and calcium lactate) and 2 calcium not linked, (glucose + $CaCl_2$ and glycerol + $CaCl_2$). Crystals were observed in all conditions since the third incubation-day in semisolid media. The largest produced crystals were observed in glycerol + $CaCl_2$ medium (228±43nm) in contrast to calcium-acetate, glucose + $CaCl_2$ and calcium-lactate media. Diffraction data show that calcite was the prevalent polymorphism in all conditions. The four conditions influenced the crystallinity and the quality of the crystal formed. Crystallite size calculated by Scherrer's equation shows a final crystal grow of 36, 24, 26 and 30 nm for glucose, glycerol, lactate and acetate respectively. Crystallinity index was calculated by mean calcite diffraction peak integration, the highest IC at 9 days was 91% in glucose media. EDS quantification analysis evidenced presence of Ca, O and C as main elemental components, however FTIR analysis show amides, phosphates and sulfur functional groups presence. Those are functional groups constituents in biofilm and exopolymer proteins, amyloids, phospholipids and phosphates sugars [7]. This material surrounds the obtained crystal in all carbon sources, nevertheless, biofilm does not modify mineralized composition. The thermogravimetric analysis showed a difference in the amount of amorphous material produced in all carbon sources, results show that crystals obtained in glycerol carbon sources have the major weight loss (~20%) starting at 296.5 °C. Weight loss indicates organic matter decomposition and probably amorphous calcium carbonate melt point. In all conditions, a second weight loss is observed at 700°C-800°C, which is attributed to $CaCO_3$ decomposition to CaO. Finally, it was determined that *B. subtilis* produces more calcium carbonate crystals, 85% mineralized calcium, in calcium-acetate medium. This work demonstrates that use of different carbon sources coupled to *Bacillus subtilis* 168 can be used as a biological system that can produce calcite with different crystallinity and micromorphology characteristics, that implies an advantage for the

applications of $CaCO_3$.

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