Phase II/2021

***Formulation and optimization of the bio-bacterial product based on selected bacterial cells and adjuvants (agro-industrial by-products*)**

Abstract

The activities were the following: Act 2.1. Selection of bacterial strains specialized in carbonatogenesis (part 2) (CO); Act 2.2. Optimization of cultivation factors to improve the carbonatogenesis capacity of selected strains (CO); Act 2.3 Elaboration of procedures/methods for characterization of construction materials embedded with bacterial cells (Part 2) (P1); Act 2.4. The influence of adjuvants (agro-industrial by-products) on the activity of the bacterial bioproduct and the optimization of its activity to increase the durability of construction materials (CO and P1); Act 2.5. Testing the survival capacity of bacterial cells included in construction materials supplemented with adjuvants (agro-industrial by-products) (CO and P1); Act 2.6. Dissemination of project results (CO, P1).

In phase II /2021, the testing of bacterial strains from the ICEHIM Microbial Collection was continued by culturing on media to facilitate the precipitation of calcium carbonate. Since a characteristic of microorganisms that precipitate calcite is ureolysis, the selected bacterial strains were tested for the ability to secrete the urease enzyme by culturing on specific media. The positive reaction consisted in changing the color of the culture medium, from yellow/orange to pink. At the first test, more intense coloration was noticed for the three *Bacillus* strains, which were cultured on media with and without urea, nickel salts, calcium ions, etc., to highlight the enzymatic activity of urease. The bacterial strains were cultured on liquid media in agitated flasks, and the deposits obtained from filtration were analyzed by scanning electron microscopy (SEM), optical microscopy and Fourier Transform infrared spectroscopy (FTIR). Microscopic observations of the samples showed the formation of crystals (mostly amorphous) mixed with cellular biomass. FTIR analysis of bacterial biomass samples showed the presence of a specific peak in the range 1405-1415 cm^{-1}, attributed to the asymmetric stretching vibration of the carbonate group.

The corroboration of the obtained results with those provided by the literature led to the selection of the *Bacillus subtilis* strain for the following experiments. The selected strain was cultured at different values of the physical-chemical parameters of cultivation, namely, at a temperature of 20, 30, 40, 50^{o}C, pH values 6, 7, 8, 9, 10, and reaction time (2, 3 and 4 days). The optimal conditions for cultivation and precipitation of calcium carbonate were found to be, temperature of 30^{o}C, initial pH medium of 7.0 and the duration of the process of 3 days.

Several experiments were performed to incorporate bacterial cells from *Bacillus subtilis* into the mortar matrix, by testing different concentrations of bacterial suspension (DO_{600nm} =0.996; DO_{600nm} =0.421; DO_{600nm} =1.339). Mechanical strength, water absorption and setting time of samples were recorded. Mostar samples were broken at 2, 7, 28 and 56 days and analyzed by SEM and FTIR. SEM images showed the specific presence of acicular crystals of ettringite and hexagonal crystals of hydrated calcium hydroxide. Optical microscopy highlighted the viability of bacterial cells in cement. The bands in the range 1070–1085 cm^{-1} corresponding to the CO_3^{2-} group were highlighted. The incorporation of the bacterial suspensions in the mortar led to the following results: decrease of the porosity values and implicitly an increase of the compactness of the mortars with bacteria; a slight increase in their apparent density, compared to the standard mortar; increasing the compressive strength of mortars in which the bacterial bioproduct was included.

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