D3.35 - BIOREACTOR DESIGN

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Document History			
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CONTENTS

1 - DOW OBJECTIVES

The activity is targeted to the design of a proposed bioreactor that can be introduced into the productive process of the cement industry and that involves the technologies developed during the investigation. The design will take into consideration the feedback originated from the on-site demonstration real case of a cement factory and shall be eco-efficiency in its new production process

The design of the bioreactor for Eco-cement trial production is a consequence of the results achieved in previous deliverables:

2 – IDENTIFICATION OF MAIN PARAMETEERS

2.1 - Suitable microbial sources for enzyme production (DL3.31)

According to the experimental data, the test results qualify *Sporosarcina pasteurii* as the most suitable bacteria for the Eco-Cement process.

From DL3.32

2.2 - *Influence of Temperature*

The incubation temperature has an influence on the urea conversion rate. Based on the results from the investigations on pure urease (D3.31 section 5) and the results from the recorded growth curves by ICBVC, the system could be described as dependent. As *S.pasteurii* generates energy by the urease reaction, it benefits of a high urease activity at warmer conditions. The investigated temperatures from 20 °C to 38 °C gave higher urease activities for increasing temperatures. The bacterial growth accelerates, when the bacterium has enough energy. We measure more ammonium per time if the urease activity is higher and also if we have more urease producing bacteria cells. So it is a combination of favourable growth condition (T) and higher urease activity. We observed the most favorable growth conditions in terms of incubation temperature at 30 °C.

2.3 - Influence of pH

The pH has a clearly impact on the bacterial enzymatic activity, the maximum values for the conductivity and ammonium ions were observed for the pH ≤ 10.

2.4 - Influence of Urea

Compared with expensive high pure urea purchased from Sigma (SU), fertilizer urea (FU) showed no differences in the recorded growth curves of *S.pasteurii*. Therefore in the process we substitute SU with FU. Higher the urea concentration, higher the urease activity with an optimal ratio for 1M to 1.33 M concentration. In order to save money and to lower the environmental impact we decide to use a 0.33 M of Urea being this concentration sufficient to the aim of the

ecocementation process. This concentration should be increased in the case of a CKD containing more than 20% of free calcium.

2.5 -Influence of bacterial density

The bacterial cultures of *S. pasteurii* in the late exponential phase or the early stationary phase led to a immediately precipitation of the CaCO3, when a calcium source reach these cultures. This phenomenon is due to the favorable conditions induced by the metabolic activity of these ureolytic bacteria. In these bacterial phases, the ionic specimens are in equilibrium, almost all the urea being hydrolyzed and the nutrient medium metabolized, and therefore, a chemical induced $CaCO_3$ precipitation may be favored when a Ca^{2+} source is added. The fact that the initial biomass of bacterial culture drastically decreased after adding the calcium source, confirm that *S. pasteurii* behaves as a nucleation site for the $CaCO_3$ formation.

A linear correlation between bacterial cells in solution and urease activity was observed, but till a maximum bacterial initial concentration of 5.60E+07 CFU/mL. It seems that there was a limit for improvement of the urease activity caused by more cells in solution.

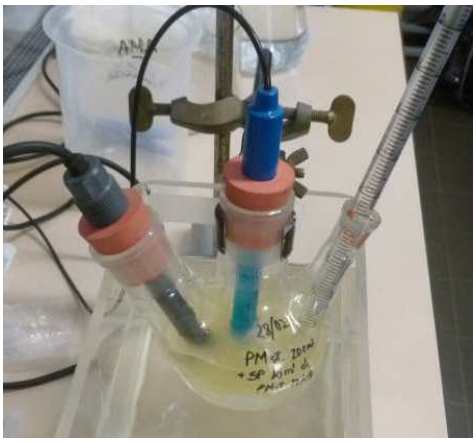
*2.6 - Nutrient alternatives**(DL3.33)***

The most suitable alternative source for the production of the biomass necessary for the processis **Permeate**or **Whey** (carbon source frommilk factorydairy waste) which is used as such due to the lowest protein concentration (1.4 g/L), producing about 40% of $NH4^+$, with respect to the reference value obtained using 20g/L of CASO nutrient medium.

3 - MICROBIAL PROCESS FOR ECO-CEMENT BIOMASS PRODUCTION (DL3.34)

***SBI** - Starting Bacterial Inoculum*

200 ml of Permeate + the urea source coming from agricultural sector (FU 2%) was inoculated with 10 mL of a culture of *Sporosarcinia pasteurii* (in late exponential phase grown in sterile medium), with a value of the conductivity 28.6 mS at the moment of the use.



Starting SP culture

BP - *Biomass Production*

After 2 days The whole SBI is poured into the Bioreactor containing 2 liters of Permeate. The bacteria are let to growth for 2 days, checking the conductivity, ATP and total ammonium, until their cell number is about 10^8/mL.

SBI of SP sown into the Bioreactor (2 liters)

BS - *Biomass Storage*

Immediately after $T_{zero+24\ hours}$, the bacterial biomass developed in the 2L bioreactor, was harvested by centrifugation (7000 rpm x 12 min) and the pellet suspended in 500 mL of the Permeate culture broth. This concentrated culture broth, contains a biomass of about 5.0x 10^8 CFU/mL, was mixed with 24 g of dry PAV[1] and after homogenization distributed in plastic container (5 g each) and let to dry. The approximate biomass concentration present in the dry matter is about 1 x 10^9 CFU/g PAV.

Centrifugation of SP and mix with PAV for dry storage

[1]PAV – is the final product from the FERPODE Project, a ripen and dried poultry manure bio stabilized and hygienically safe.

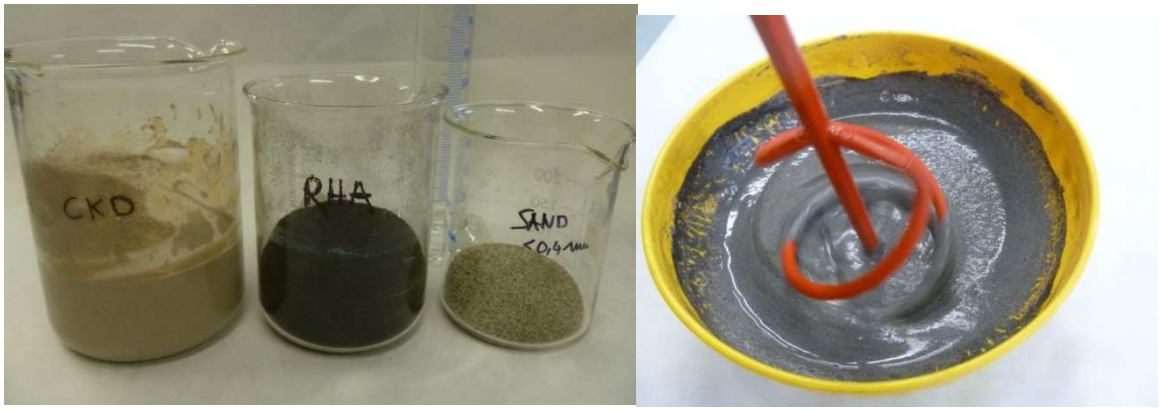
MIC *- Microbial Inoculum for bio-Cementation (see DL. 3.33)*
In order to guarantee a good bio-cementation process together with the other components (binder and aggregates) we need a bacterial biomass, which should be simple to manage and able to produce the necessary carbonate through its metabolic activity of urea hydrolysis in environmental conditions.
This MIC is composed by a culture of *Sporosarcina pasteurii* grown up to exponential phase in Whey medium and after centrifugation mixed with PAV (2:1) and let to dry at room temperature (BS) .
This dry mix can be adjusted with a concentration of SP cells varying from about 10^8 to 10^9 for gram.

4. – ECO-CEMENT ON SITE WORKING PROCESS

A. **Revitalization of the Biological Mass** - In a plastic container add the necessary amount of the BS (Biomass Storage) components and 0.5 liters of Urea solution and incubated, about 3 hours (until stationary or exponential growth phase is achieved) in a water bath (at 28-30°C) with gentle stirring (see video on the web site). If a water bath is not available, the water bath temperature could be adjusted mixing boiling and cold water. Once revitalized the solution containing PAV and SP cells must be gently blend and filtered by a thin mesh strainer. The clear liquid solution obtained contains the needed living cells of SP to add to other components.

Biomass revitalization

B. **Cement** - In a concrete mixer insert the CKD, RHA, and Sand components and mix thoroughly for few minutes, then add the urea solution necessary to create a fluid paste (9 liters) with constant amalgamating and slow action.
C. **Bio-cement** – Without interruption in the mixing add the biological mass (A) after its revitalization and filtering.



Blending of the Ecocement components: CKD; RHA; Sand; Urea Solution and Biomass

5. – ECO-CEMENTS PRODUCTS

I. **Tile** – Pour the mix in adequate formworks and wait until seasoning/curing (28 days).

II. **Plaster –** apply, by a trowel, a layer 1 cm thick on a wall (1 x 1 m).

III. **Bedding mortar -** apply, by a trowel, a layer 1 cm between two bricks in succession in order to build a wall of 5 bricks height and 4 bricks length 2 bricks width.

Ecocement products: I)Tile – II) Plaster – III) Bedding mortar

6 –MATERIAL AND COSTS ESTIMATION FOR THE ECO-CEMENT DEMONSTRATION

STANDARD RECIPE = 1 – Aggregate (sand)
3 - Binder (CKD+RHA)
1 – Water

STANDARD CUBE			1%	2%	5x5x5=125cm^3
CKD (g)	RHA (g)	Sand (g)	dry PAV+SP (g)	FU (g)	Total H2O
100	20	34	1.2	2.4	120

FOR ON SITE DEMO OF THE APPLICATION AS MORTAR

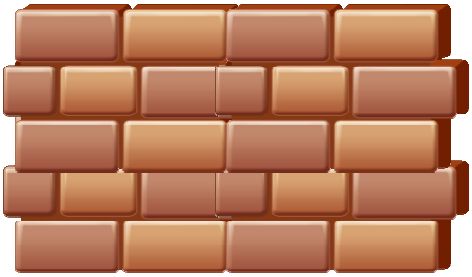
Assuming to cover with a plaster layer of 1 cm thick a surface of about 1 square meter

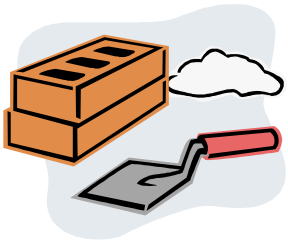
PLASTER			1%	2%	100x100x1=10000 cm^3
CKD (g)	RHA (g)	Sand (g)	dry PAV+SP (g)	FU (g)	Total H2O
8	1.6	2.72	0.096	0.192	9.6

FOR ON SITE DEMO OF THEAPPLICATION AS BEDDING MORTAR

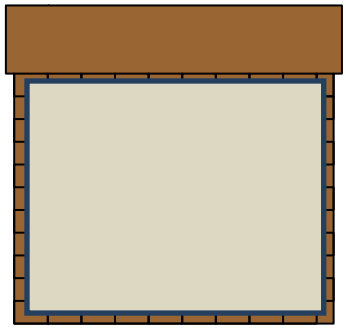
Assuming to build a brick wall (brick dimensions20x10x5cm)composed of a column of 4 bricks length 2 bricks width and 5 brick height (mortar layer thickness 1 cm)

BEDDING MORTAR			1%	2%	20x20x1=200 cm^3 x 32 = 6400 cm^3 10x5x1= 50 cm^3x 30 = 1500 cm^3 Amount of plaster between bricks in the wall
CKD (Kg)	RHA (Kg)	Sand (Kg)	dry PAV+SP (Kg)	FU (Kg)	Total H2O (liter)
3.2	0.64	1.09	0.384	0.768	3.84





Bedding mortar



Plaster

On the demo test site we need a water bath (30° C) – for revitalize the BM; a blender; a mixing container; a strainer, a spatula or trowel, pH strip, thermometer and a balance if the components are not pre-weight. The plaster board and the bricks should be present on the site.