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## CHARACTERIZATION AND PROPERTIES OF A MICROBIAL EMULSIFIER PRODUCED FROM CRUDE GLYCEROL

Macarena Rulli[1], Analía Alvarez[2 3], Luciana Del Gobbo [1], María Fuentes[2], Verónica Colin[2]

*[1]Universidad Nacional de Tucumán, Facultad de Bioquímica, Química y Farmacia. [2]Planta Piloto de Procesos Industriales Microbiológicos. [3]Facultad de Ciencias Naturales e Instituto Miguel Lillo.*

veronicacollin@yahoo.com.ar

Bioemulsifiers (BE) are amphipathic molecules used in the bioremediation field due to the role in emulsification, solubilization, and removal of hydrophobic compounds from the environment. The ability of a spore-forming bacterium to produce BE using crude glycerol as a cheap feedstock was previously detected. In the current study, partially purified BE was characterized. The ability of the microbial product and of two commercial synthetic agents such as sodium dodecyl sulfate (SDS) and Triton X-100 (TX-100) to emulsify hydrophobic substrates was also comparatively evaluated. Culture supernatant containing BE was filtered through a dialysis tubing cellulose membrane (Typical molecular weight cut-off = 14,000 Da). The concentrate obtained was then used as BE source, and subjected to hydrolytic treatments with proteinase K (30 U mg-1 at 37 °C for 4 h), commercial lipase from *Candida rugosa* (100 U mg-1 at 37 °C for 1 h), and acid hydrolysis (10% HCl at 100 °C for 10 min) in order to estimate the role of peptides, lipids and sugars on the BE nature. The biodegradability of BE, SDS and TX-100 was assayed using the BOD/COD ratio, with BOD and COD as the biological and chemical oxygen demand, respectively. BOD and COD parameters were determined according to the Standard Methods for the Examination of Water and Wastewater. Finally, it was evaluated the emulsifying ability of the three agents on hydrophobic substrates (kerosene, toluene, chloroform, chlordane pesticide and vegetable oils), determining the emulsification index for each substrate after being left to settle for 24 h (E24). All hydrolytic treatments significantly reduced the BE activity on the kerosene, suggesting that the microbial product could have a protein fraction as well as sugar and lipid fractions. On the other hand, a virtually negligible BOD/COD ratio was detected for SDS and TX-100 (0.070-0.172), confirming the extremely low biodegradability of these synthetic products. However, BOD/COD ratio was significantly increased for BE (0.386), so confirming its biodegradable character. Finally, a differential performance of the BE, SDS, and TX-100 to emulsify the hydrophobic compounds was detected: a similar performance of the three agents to emulsify substrates as kerosene and toluene were detected, with E24-values of 61% and 62%, respectively. However, chloroform was only effectively emulsified by the BE, with an E24-value increased 4-fold compared to those detected for the synthetic agents. While SDS had poor ability to emulsify a pesticide such as chlordane (E24 = 18%), the BE and TX-100 were optimal emulsifying agents for this substrate, with similar E24-values between them (61%). Finally, only the BE was able to emulsify vegetable oils as sunflower, canola, and grape, with E24-values that ranged from 38% to 51%. These results could encourage the application of a biodegradable microbial product to achieve the effective removal of hydrophobic pollutants, without detriment to the environment. Supported by PICT 2015 Nº 0297 and PIP 0372.





MS-011

## SYNTHESIS OF BIOFILM, POLYHYDROXYALKANOATES ACCUMULATION AND PROTEOLYTIC ACTIVITIES BY *Bacillus subtilis* subp. *spizizenii* USING GLYCEROL AS CARBON SOURCE

Mirta E. Galelli[1], Silvia S. Miyazaki[1]

*[1]Universidad de Buenos Aires. Fac Agronomía. Área de Agroalimentos. Av. San Martín 4453. CABA.*

mgalelli@agro.uba.ar

*Bacillus subtilis* subsp *spizizenii,* a PGPR organism, synthesis biofilm at the liquid-air interface. Biofilm is a survival strategy, giving protection against environmental fluctuations in humidity, temperature, pH and nutrient availability. These characteristics are important for its use as bioinoculant. Glycerol is an industry by-product suitable as carbon source. Yeast extract is a possible source of amino acids. Bacterium survival is benefited by the reserve material polyhydroxyalkanoates (PHAs). The objectives of this work were the production of a biofilm with sessile PGPR bacteria suitable as a biofetilizer using glycerol in a medium supplemented with yeast extract; the study of biofilm characteristics, PHAs accumulation and proteolytic activities. *Bacillus subtilis* subsp. *spizizenii* free of plasmids was used. The culture media were basal salt medium supplemented with amino acids or with yeast extract (YE) (0,2; 0,5 and 1%); with or without 1% glycerol. The biofilm formation was determined in static conditions at 96 h of incubation at30 °C. Bacterial growth was measured as optic density at 600nm (OD). Protease activity was determined with azocasein. The PHAs were determined by crotonic acid formation. Data were analyzed by ANOVA test. *B. subtilis* needed specific amino acids (glutamic acid, aspartic acid or lysine but not tryptophan) to synthesize biofilm (BF) using glycerol. For glutamic, the minimum needed was 0,07%. The bacterium formed biofilm using YE (without glycerol) at concentrations higher than 0,5% (0,062 mg BF/ml). With 1% glycerol and 1% YE, biofilm formation increased (423%) and planktonic cells decreased (810%). Biofilm robustness was greater with glycerol. At 0,2% YE, with or without glycerol, there was not biofilm synthesis, glycerol increased 220% plancktonic growth. High proteolytic activity (around 90 U/ml) was observed in the medium with only YE. With glycerol there was proteolytic activity only when YE was higher than 0,5%, being maximum for 1% YE. PHAs accumulation in biofilm sessile cells was lower for YE 1% than for 0.5% (29.3 and 65.1 mg PHAs/mg BF respectively). With glycerol, PHAs concentrations in biofilm were similar for both yeast concentrations (around 8 mg PHAs/mg BF). The maximum PHAs accumulation (756 mg PHAs/OD) in the planktonic cells was with 1% YE and 1% glycerol, for the others assay conditions the accumulation were similar (around 185 mg PHAs/OD). Amino acids were needed for the synthesis of a biofilm by *B. subtilis* using glycerol as a carbon source. Due to the bacterium proteolytic capacity, yeast extract was a suitable source of the amino acids. *B. subtilis* accumulated PHAs in the sessile cells of the biofilm. This results indicate that a basal salt medium with 1% Glycerol (as a carbon source) and 1% Yeas extract (as a source of amino acids) was suitable for the production of a robust biofilm by *B. subtilis*, that accumulated PHAs, characteristics suitable for its use as biofertilizer.