Exopolymeric matrix extraction from biofilms grown in unsaturated zone in Managed Aquifer Recharge system

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INTRODUCTION

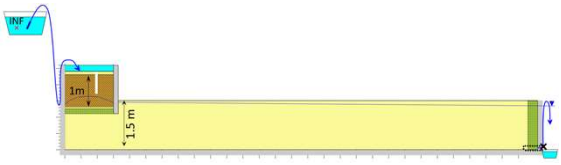
Biofilms play an important role in environmental matrices, as they affect several pollution removal processes, such as biosorption, bioaccumulation, redox immobilization, and organic matter degradation. Biofilms are aggregates of microorganism communities, attached to a surface, and encased in a self-synthesized **EPS (Extracellular Polymeric Substances)** matrix consisting of water, proteins, carbohydrates, and extracellular DNA. EPS yields cell protection, biofilm structure, nutrient trapping, water retention, and genetic exchange. **EPS and microorganisms diversity are essential to understand the behaviour of the biofilm in relation to environmental variables and pollutants removal.**

AIM

Establish an EPS extraction methodology using cation exchange resin (CER) from biofilms to avoid sample contamination

MATERIAL AND METHODS

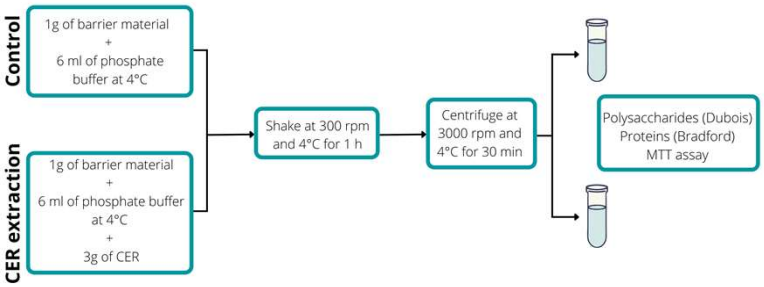
Sampling



Samples were taken from an experimental site consisting of **six pilot-scale MAR systems** (**rbMAR**), recharged with a WWTP effluent (Valhondo et al., 2020). Recharge water flows across a reactive layer located at the basin bottom. Samples were taken at 4 depths along 3 months of operation.

The reactive barrier consist of sand (50%), vegetal compost and wood chips (32%), biochar (10%) and clinoptilolite (8%).

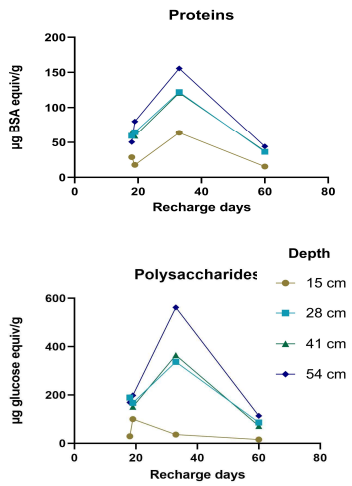
EPS extraction and quantification



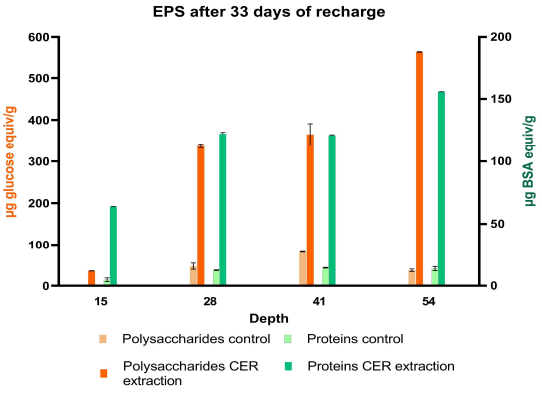
The EPSs were extracted by adding CER (as a control, the same process was carried out without the resin). Exopolymers (proteins and polysaccharides) were quantified. After EPS extraction, the MTT assay (Wang et al., 2010) was performed to measure enzymatic activity. Following MTT protocol, an initial bacterial culture was performed using reactive barrier material as MSB medium.

RESULTS

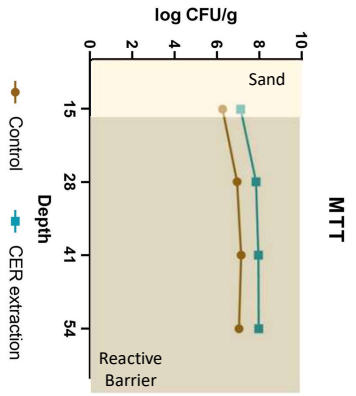
EPS content along 60 days of recharge



EPS content



Metabolic activity after extraction



The addition of the Cation Exchange Resin enhances the extraction of EPS without contaminating the samples. After CER extraction samples did not show differences in their metabolic activity. The maximum peak of EPS production was observed after 33 days of recharge. EPS content was observed to vary with depth (the highest proportion between 28 and 54 cm deep). We are currently studying the relationship between biofilm growth and water quality.

REFERENCES

Frølund, B., Palmgren, R., Keiding, K., & Nielsen, P. H. (1996). Extraction of extracellular polymers from activated sludge using a cation exchange resin. *Water research*, *30*(8), 1749-1758.
Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. T., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical chemistry*, *28*(3), 350-356.
Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical biochemistry*, *72*(1-2), 248-254.
Valhondo, C., Martínez-Landa, L., Carrera, J., Díaz-Cruz, S. M., Amalfitano, S., & Levantesi, C. (2020). Six artificial recharge pilot replicates to gain insight into water quality enhancement processes. *Chemosphere*, *240*, 124826.
Wang, H., Cheng, H., Wang, F., Wei, D., & Wang, X. (2010). An improved 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) reduction assay for evaluating the viability of Escherichia coli cells. *Journal of microbiological methods*, *82*(3), 330-333.

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