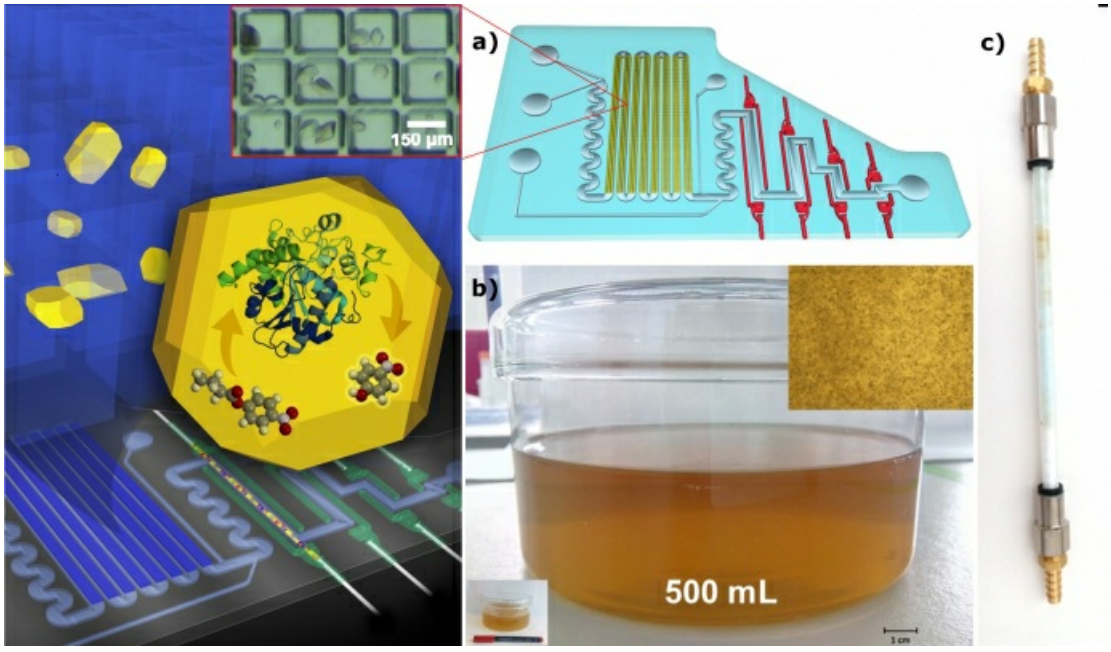
Poster Presentation

MS009.P14

Biotechnological application of enzyme crystals, from microfluidic to batch production.

Jose Antonio Gavira Gallardo[1], Cristobal Verdugo-Escamilla[1], Isaac Rodriguez-Ruiz[2], Mayte Conejero-Muriel[1]
[1]*Lec, Iact, Csic-Ugr, Armilla, Spain,* [2]*CEA, DEN, DMRC, SA2I, F-30207, Bagnols-sur-Cèze, France*
E-mail: jgavira@iact.ugr-csic.es

Biocatalysts make use of the versatility, selectivity and specificity of enzymes to catalyze a variety of processes for the production of relevant compounds under mild conditions. One of the most common strategies to extend the lifetime under extreme conditions and to increase the efficiency of enzymes is their immobilization in different materials or the auto-immobilization by cross-linking. In this context, cross-linked enzyme crystals (CLECs), yet proven to be a better solution to enhance catalyst lifetime, recoverability, etc. when compared with cross-linked enzyme aggregates (CLEAs), have been almost abandoned [1]. Cross-linked enzyme crystals (CLECs) have been neglected although they are proven to be a better solution to enhance catalyst lifetime, recoverability, etc. when compared with cross-linked enzyme aggregates (CLEAs) [1].
We have recently demonstrated that the use of CLECs-based microreactors, shows unprecedented self-storage capability and stability as compared to standard biosensors, which cannot be stored for long periods due to quick denaturation of the enzymes (with lifetimes of weeks in the best case) [2]. We have further extended this concept by combining an enzymatic (lipase) microreactor, operating in continuous mode, with an optofluidic detection system [3] (Figure 1.a). The use of enzymatic catalytic reactions under microfluidic flow conditions reveals a promising technology with a number of strategic advantages such as the dramatic improvement of surface/volume ratios or enhanced energy consumption and mass transport, which are fundamental for the fabrication of novel biosensor systems based on CLECs.
In this work, we have explored the use of different gels in order to control nucleation and growth of lipase crystals while providing a diffusion mass transport environment for the incorporation of cross-linkers inside the crystals, thus avoiding any osmotic shock. The production of Reinforced Cross-linked Lipase Crystals (RCLLCs), recovery, and enzymatic characterization are shown in this communication. RCLLCs have been used to pack a 10 cm chromatographic column, a scale-up representation of the microfluidic approach that operates in continuous flow (Figure 1.b). The characteristics and main features of both systems, micro and macro scale flow systems, are shown and compared.
[1] Brady, D. et al. (2009). J. Biotech. Letters, 31, 1639-1650.
[2] Conejero-Muriel, M. et al. (2015). Lab Chip, 15, 4083-4089.
[3] Conejero-Muriel, M. et al., (2016). Anal. Chem. 88, 11919−11923.



Keywords: CLECs, RCLECs, lipases