**Organophosphate pesticide degradation in a continuous biocatalytic membrane reactor**

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**Highlights**

* Continuous biocatalytic membrane reactor development (BMR) to hydrolyse organophosphates
* Significant improvement of immobilized phosphotriesterase stability respect to the free enzyme
* High organophosphate degradation (90%) by the BMR
* Long term stability of the BMR (1 year)

**1. Introduction**

Organophosphates (OPs) are highly toxic compounds used as pesticides and nerve agents. The devastating effects, reported in different studies, on the environment and human health indicate a serious scenario for both instantaneous and long terms effects. Bio-based strategies for OPs degradation seem the most promising solutions, particularly when extremophiles enzymes are used. These systems permit OPs degradation with high efficiency and specificity under mild conditions. However, as frequently observed, enzymes can easily lose activity in batch systems, so that a strategy to improve biocatalyst stability is highly needed, in order to develop continuous systems. In this work an extremophile enzyme, able to hydrolyze organophosphates, was immobilized on functionalized polymeric membranes to develop a continuous biocatalytic membrane reactor (BMR).

**2. Methods**

Commercial flat-sheet polymeric membranes of regenerated cellulose (Millipore), PVDF (GVS spa) and PES (GVS spa) were functionalized (Militano et al., 2016, Vitola et al 2016, Vitola et al 2018) in order to covalently bound three different mutants (called SacPox, SsoF and Sso-3M) from Sulfolobus acidocaldarius, Sulfolobus solfataricus (E. Porzio, 2007), kindly supplied by the Institute of Protein Biochemistry of the National Research Council (IBP-CNR). The obtained biocatalytic membranes were characterized by FT-IR, immunoelectron microscopy, enzyme catalytic activity and stability.The activity of the phosphotriesterase towards the pesticide paraoxon at 25 °C was evaluated measuring the appearance of the reaction product (4-nitrophenol) by a spectrophotometer (405 nm).

**3. Results and discussion**

Among the different mutants with phosphotriesterase activity tested Sso3M showed the highest specific activity (10.0 ± 0.06 U/mg) in comparison to SsoF (0.65 ± 0.07 U/mg) and SacPox (0.34 ± 0.04 U/mg), respectively.

Sso3M showed the highest specific activity also when immobilized on hydrophilic membranes (PES and RC membranes) respect to hydrophobic (PVDF), giving the highest retained specific activity when immobilized on RC membranes.

The concentration of the immobilized enzyme amount which gives the highest specific activity and the best enzyme distribution on RC membranes (no enzyme aggregation observed by the in situ immunolocalization) is 0.17 (±0.03) mg/cm3. The conversion of the substrate at each passage through the biocatalytic membrane is improved up to 87%, when the residence time up to 0.96 min was increased. The stability of the enzyme in the continuous BMR was monitored for about 10 months and compared to the one of the free enzyme. The immobilized Sso3M showed high stability up to 10 months on the contrary the free enzyme is deactivated within two months

**4. Conclusions**

A continuous biocatalytic membrane reactor for the degradation of the pesticide paraoxon was developed. Under optimal conditions (residence time 0.96 min, amount of immobilized enzyme: 0.17 mg/cm3), the BMR has shown to detoxify about 90% of paraoxon (1 mM) at each passage through the biocatalytic membrane. Either in batch or in the continuous configuration, the immobilized enzyme showed higher stability than the free form up to 10 months.

**References**

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