

**Title:**

**Janus Membrane of CA/PVDF by Layer-by-Layer Electrospinning with immobilized Horseradish Peroxidase for Enzymatic Degradation of Toluene**

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**Abstract:**

Toluene is one of the most widely used industrial solvents in the world. Toluene is regarded as a carcinogen and a hazardous substance. Enzymes such as horseradish peroxidase (HRP) have been effectively used in removal of toluene. Biocatalytic membrane contactors can be used, in a process intensification strategy, as an alternative to conventional methods for toluene degradation. Indeed, membrane contactors could reduce the limitation of the efficiency of the co-substrate necessary for the activity of HRP in organic phase.

With this objective, the project focused on the preparation of CA/PVDF Janus membranes with immobilized HRP and their utilization for the conversion of toluene. The CA/PVDF membrane was developed through a layer-by-layer electrospinning method prior to surface grafting HRP covalently with glutaraldehyde (GA). GA is a reliable substance that provides excellent yields of enzyme immobilization on the support. HRP is bonded with GA through free amino groups of lysine residues of HRP.

A comprehensive characterization of obtained CA/PVDF membrane was performed by advanced analytic techniques. SEM images revealed that membrane was characterized by uniform nanofibrous and open porous structures. FTIR-ATR analysis of the membranes confirmed the successful attachment of GA to the surface of membrane. It was also observed that after immobilization of HRP, the surface zeta potential changed. Optimization of conditions (concentration of GA and HRP, time of activation of membrane) of immobilization of HRP as well the conditions (concentration of H<sub>2</sub>O<sub>2</sub> and temperature) of degradation experiments were also investigated.

CA/PVDF nanofibrous membranes were successfully designed. The efficiency of the membrane catalytic behaviour was evaluated in a novel two-phase membrane contactor module to establish baselines for conversion of toluene in optimal conditions. Immobilized HRP on CA/PVDF membrane opens unique avenues for cost-effective remediation of such mixtures towards intensification of mixed solvents and organic solvent management.