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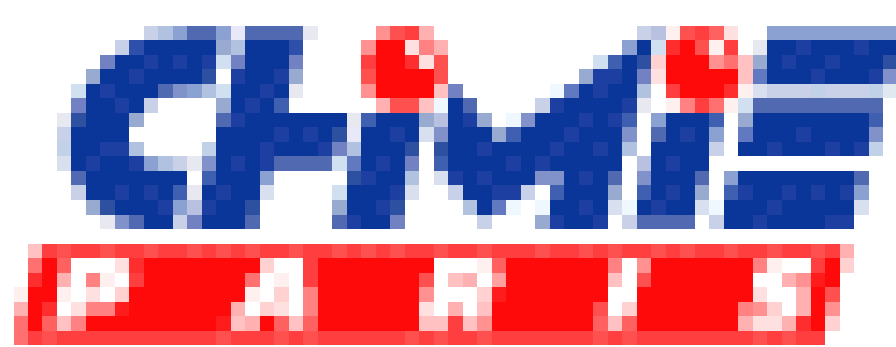
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Immobilisation of laccase from *T. versicolor* on a microfiltration membrane and application in the removal of a phenylurea pesticide



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OBJECTIVES

In the present work, laccase from *Trametes versicolor* was covalently immobilised on a hydrophilic PVDF microfiltration membrane using a site-directed immobilisation technique. The protein-grafted membrane was used in a filtration module to transform N',N'-(dimethyl)-N-(2-hydroxyphenyl)urea (2-HF), an herbicide derivative known to be oxidised into insoluble compound in the presence of laccase. The characterisation of the microfiltration membrane was performed by means of physico-chemical methods (contact angle and streaming potential measurements, XPS) throughout its modification procedure.

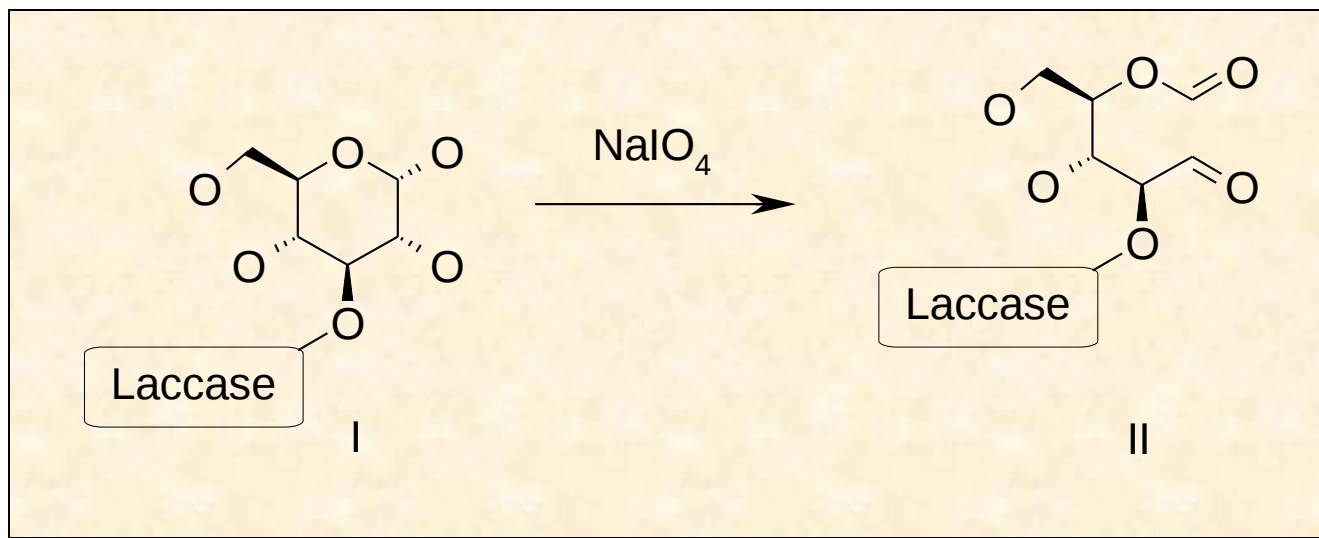
Properties of Laccases



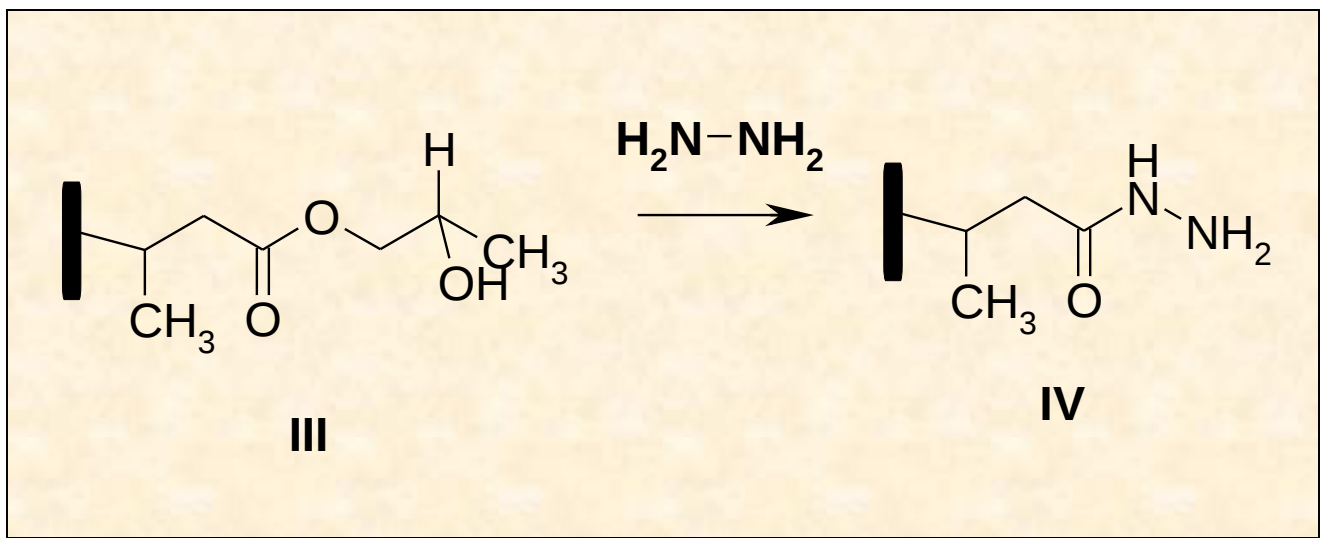
Laccases (EC. 1.10.3.2) catalyses the oxidation of various compounds, which concomitantly reducing molecular oxygen to water

Production of laccases:
① 7-days fermentation of *Trametes versicolor* (culture collection of the unité de phytopathologie, INRA, Versailles)
② Induction by 2,5-xyldine

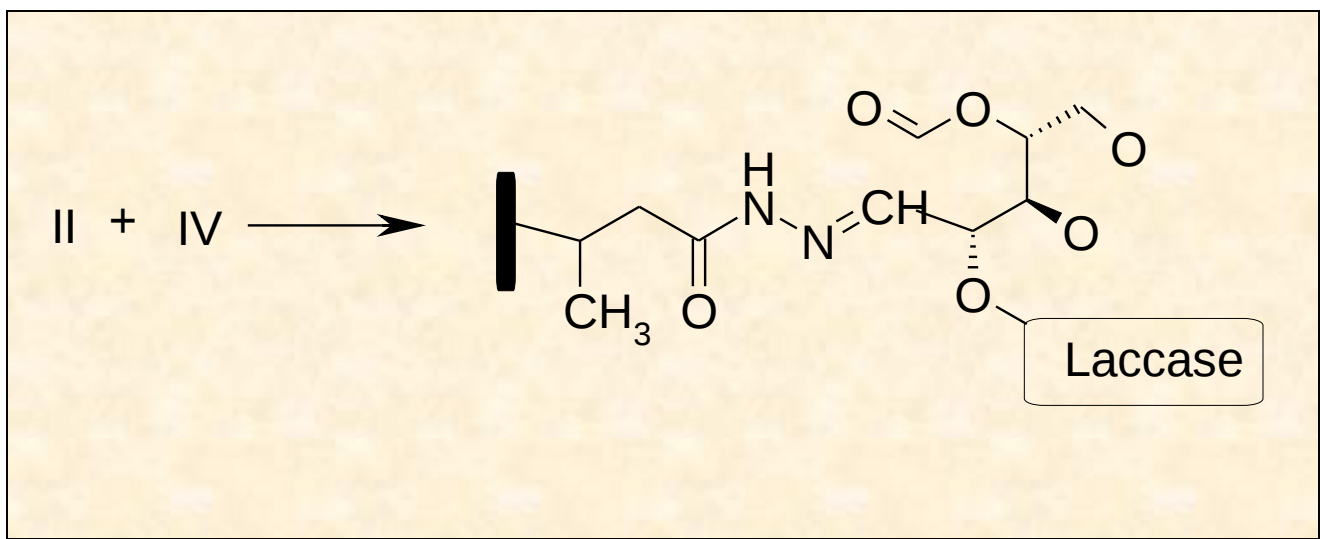
Immobilisation procedure



Step 1: Oxidation of laccase
80 % of the initial laccase activity retained

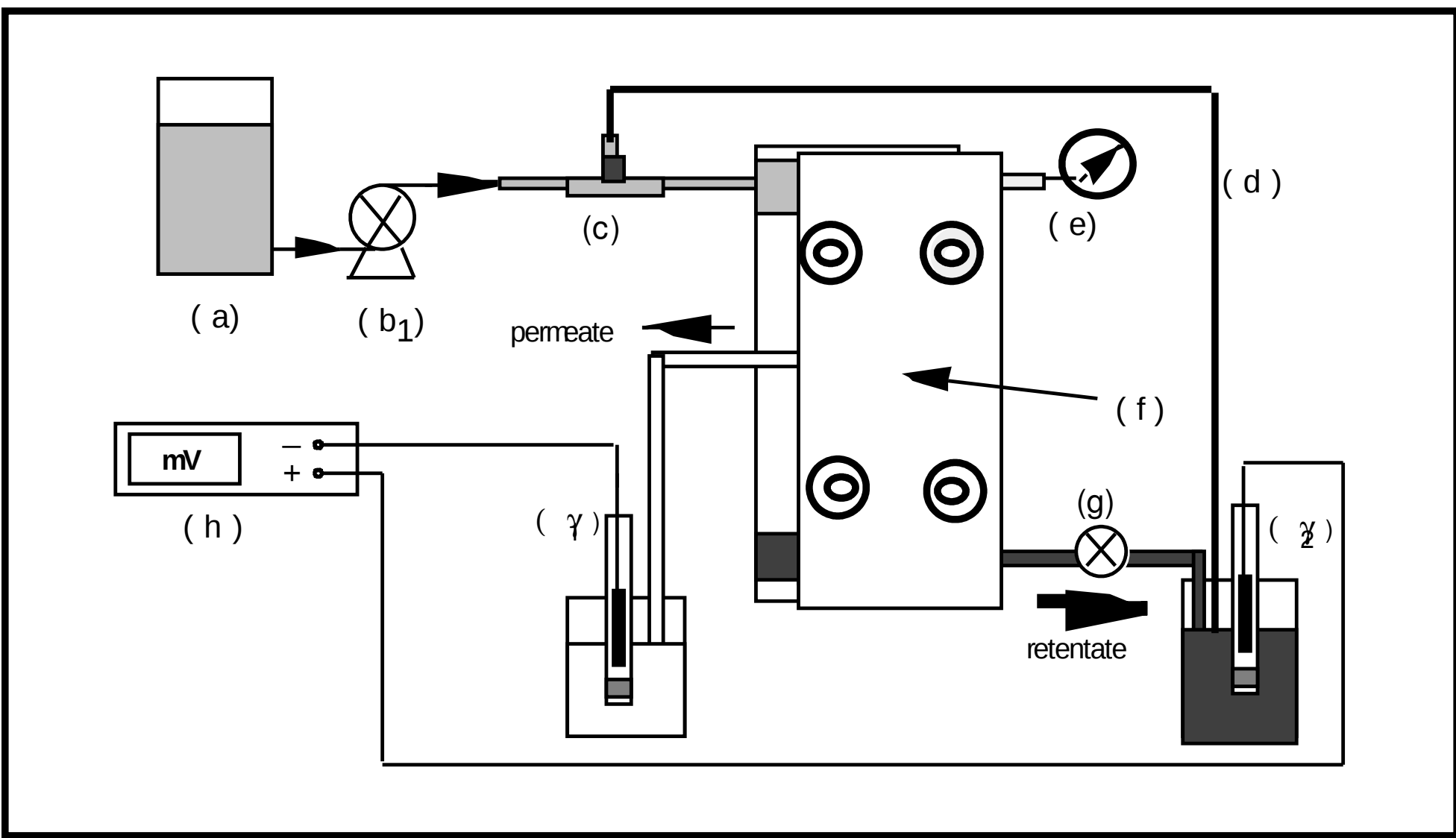


Step 1bis: Activation of the hydrophilic PVDF membrane (Millipore, Durapore 0.45µm) by hydrazine
Grafting rate: 60% from XPS measurements



Step 2: Covalent coupling of laccase to the PVDF membrane
Activity measurements in batch: 25 % recovered
Evidence for diffusion limitations in batch experiments

Experimental filtration device

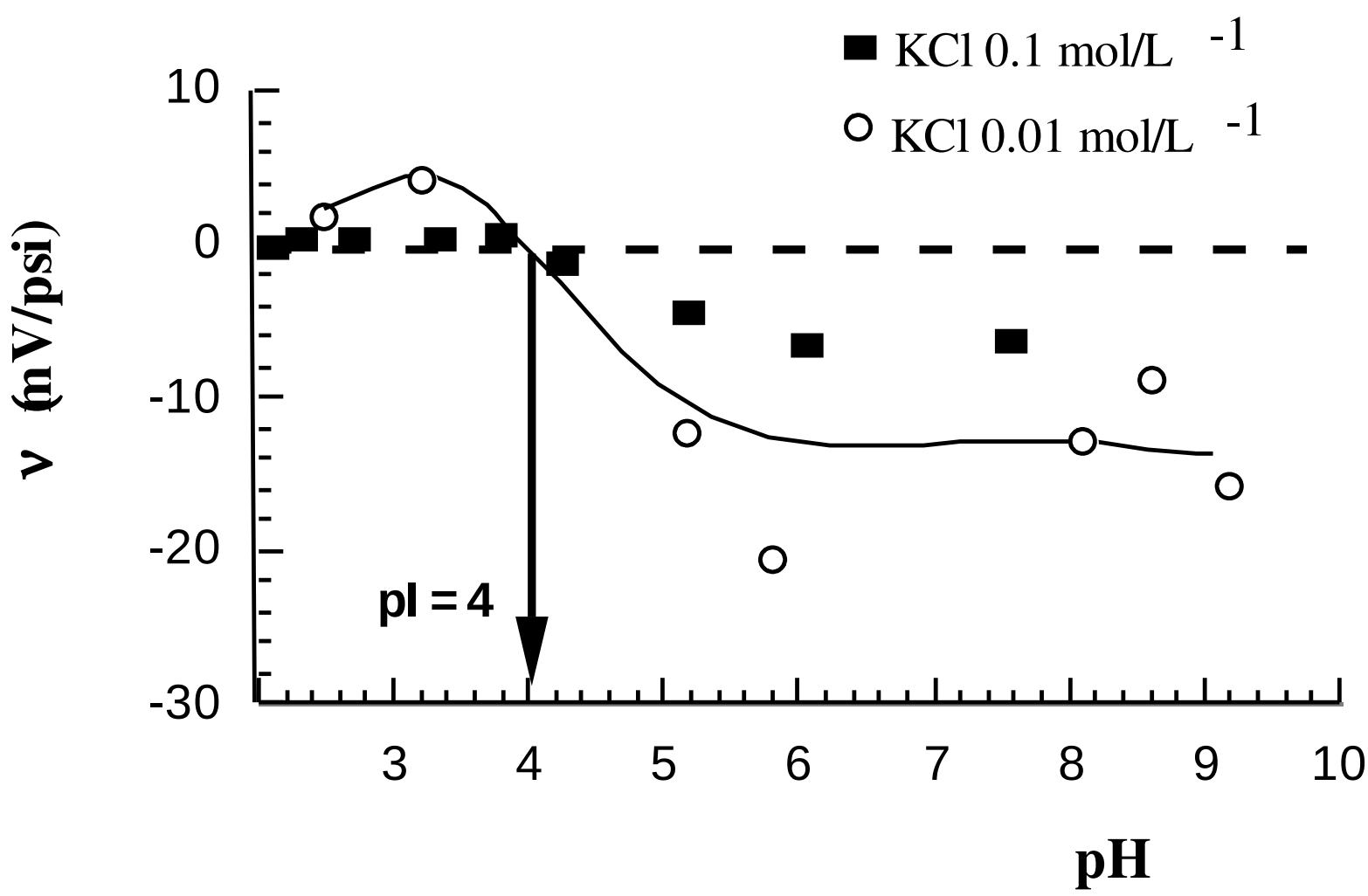


(a) reservoir
(b) peristaltic pump
(f) planar module
(γ1, γ2) saturated calomel electrodes
(i) voltmeter
(c) Vycor® fixing the junction potential

Used as shown for streaming potential measurements and without the potential measurements pieces for 2-HF filtration

RESULTS

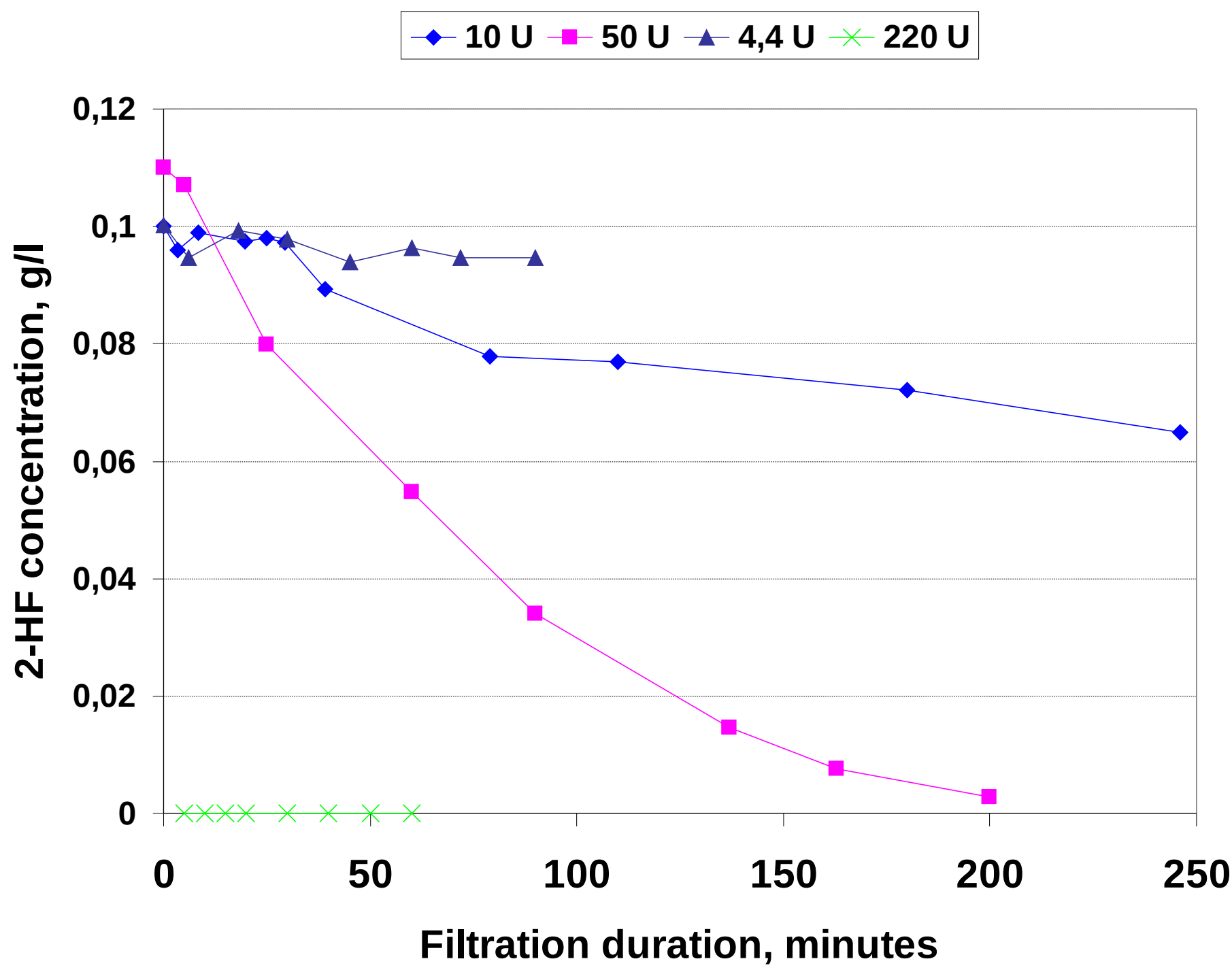
Isoelectric point determination



Streaming potential measurements

□ Isoelectric point of the grafted membrane: **pI = 4 ± 0.5** representative of the pI of the mixture of laccases

Laccase degradation efficiency



□ Removal efficiency of 2-HF in the filtrate increases with the amount of grafted laccases

Filtration module: Minitan S
120 cm² planar membranes grafted with 4.4, 10, 50 and 220 laccase activity units.
2-HF initial concentration 0.1ss g/L in acetate buffer 0.05 M, pH 5
T=22°C.

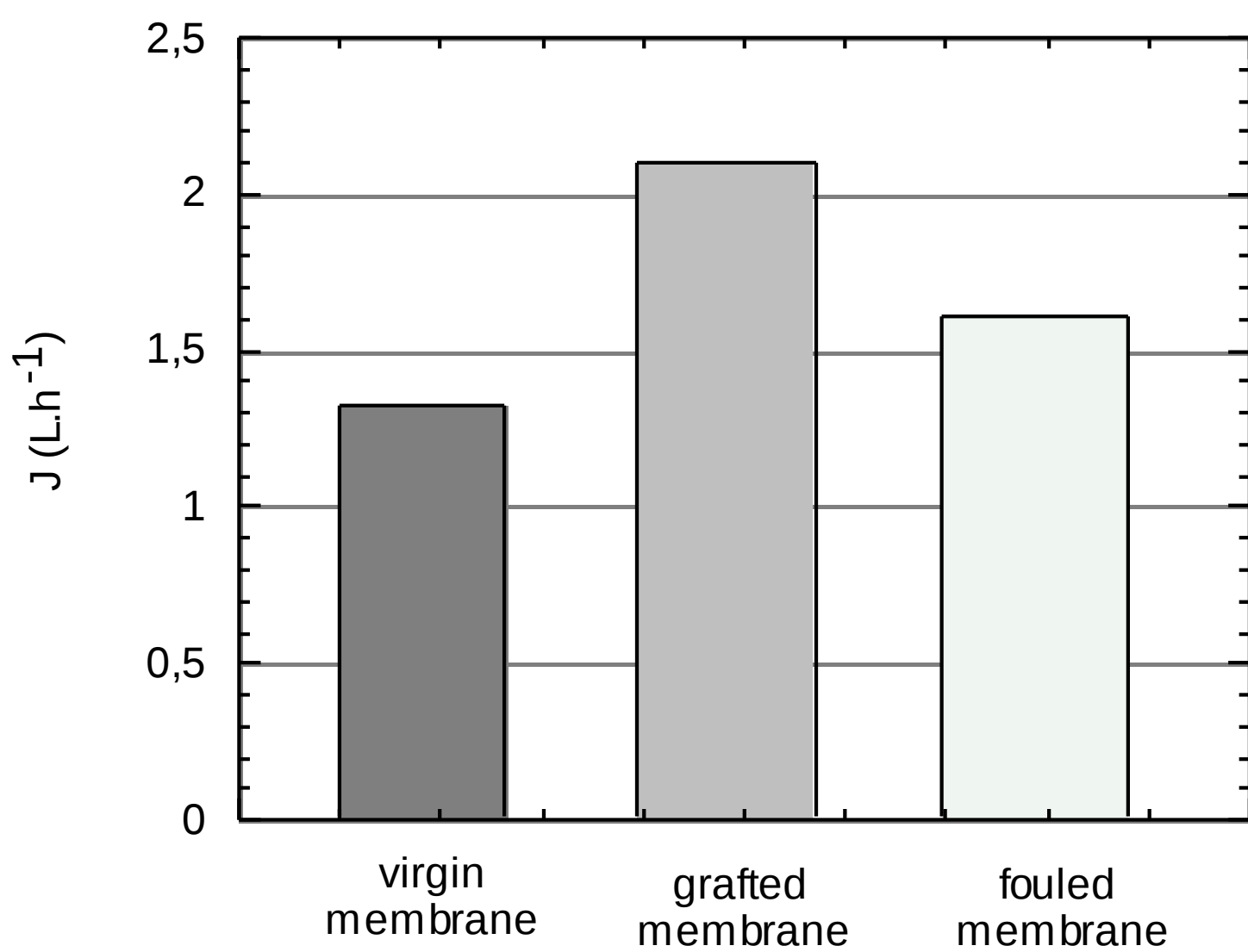
Wettability determination

Type of membrane	Contact angle, °
Hydrophilic PVDF	0°
Hydrazine activated PVDF membrane	123°
Laccase covalently grafted PVDF membrane	0°
Hydrazine activated PVDF membrane with adsorbed laccase	60°

Sessile drop contact angle technique

□ Covalent coupling of laccase to the membrane restores its hydrophilicity more efficiently than adsorption

Permeability measurements



□ Laccase coating increases the flux through the membrane
□ The precipitation of the 2-HF degradation products does not induces significative fouling

Effective membrane surface: 60 cm²
Grafted membrane with 50 laccase activity units
Fouled membrane after treatment of 200 mL of 0.1 g/L 2-HF

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