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Ultrafiltration of protein based solution: Study of membrane fouling

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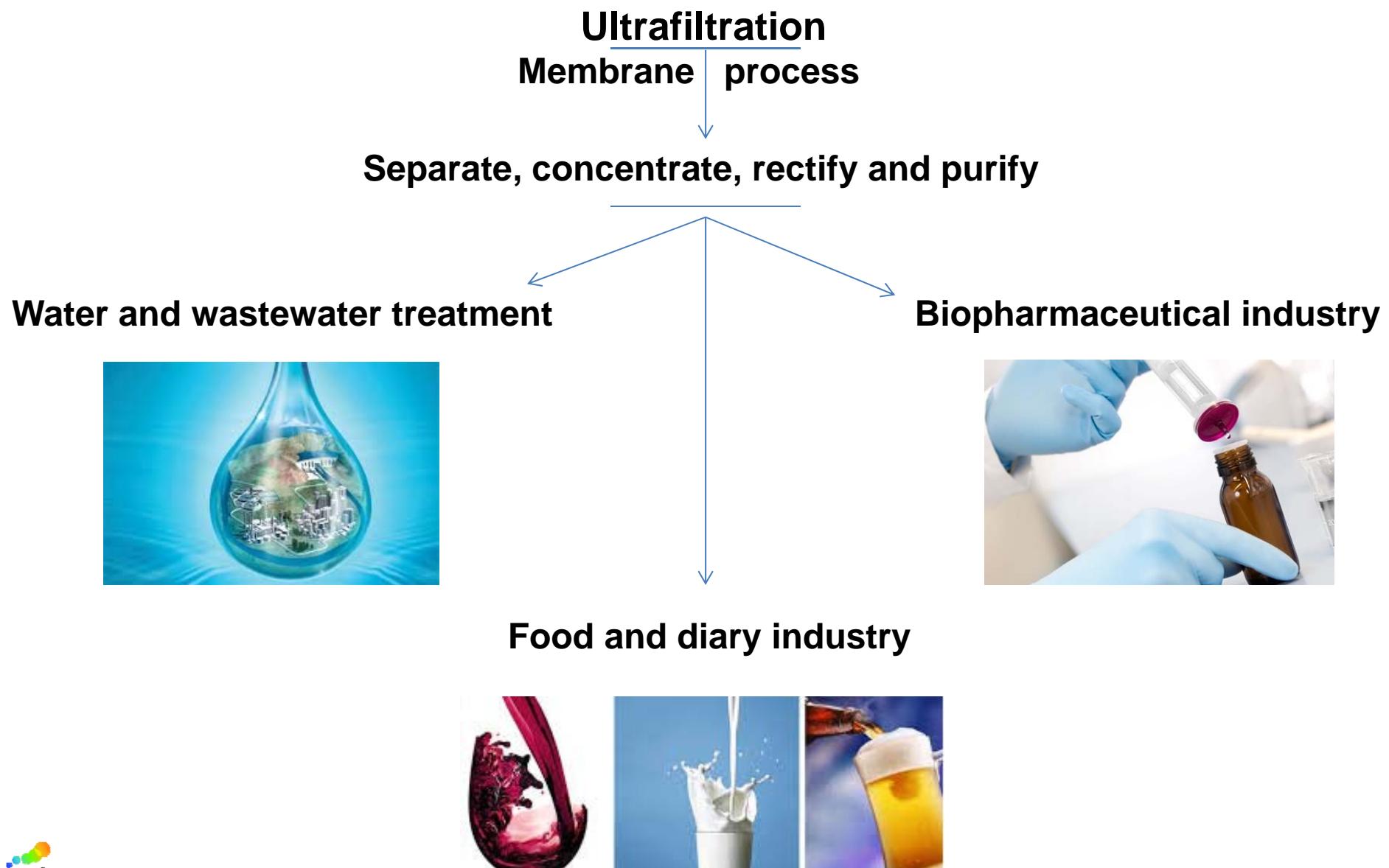
Outline

- **Introduction**
- **Study cases**

- 1. Study of interactions between biomolecules and material through cross-flow filtration - Study of fouling behavior**
- 2. Study of biological activity of the filtrated solutions**

- **Conclusions**





Part 1.

Study of interactions between biomolecules and material through cross-flow filtration

Study of fouling behavior



Hen egg white lysozyme

- enzyme with 129 amino acids
- antimicrobial activity against Gram-positive bacteria



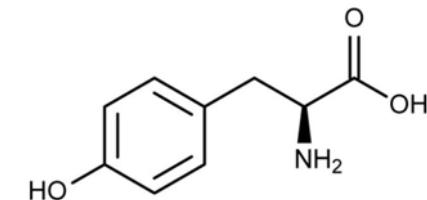
alpha-helix
 3/10 helix
 pi helix
 beta strand
 beta turn
 coil

Ribbon diagram of HEWL

Amino acid

L-tyrosine

→ Elementary constituent of Lysozyme

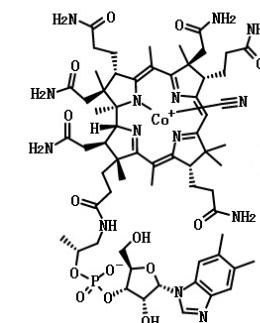


Neutral solution

Vitamin B12

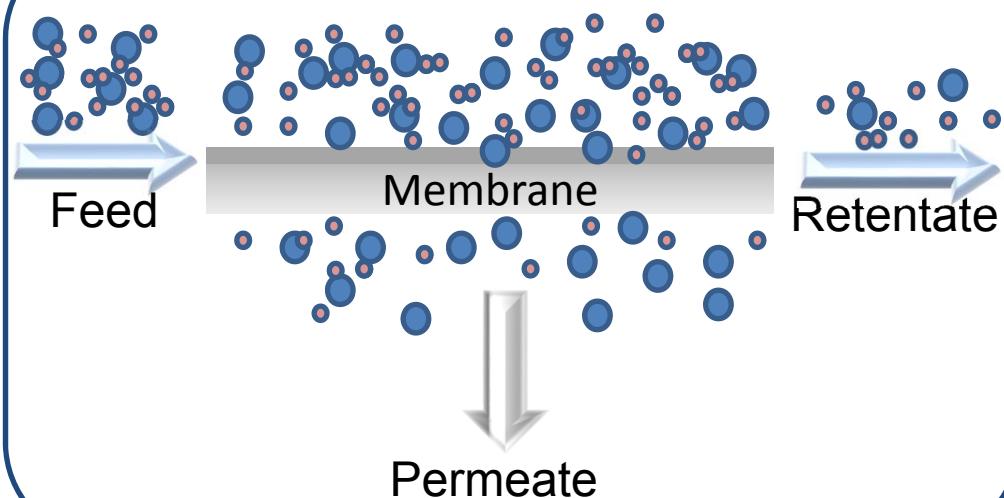
→ Used for membrane performances investigation
(Mean pore radius calculation using Nernst-Plank model)

vitamin B12



**Membrane characteristics:**

- ultrafiltration membrane
- bilayer membrane of TiO_2
- asymmetrical pore structure
- cut-off 1 kDa

**Membrane performances:****1. Selectivity performances:
rejection rate (R)**

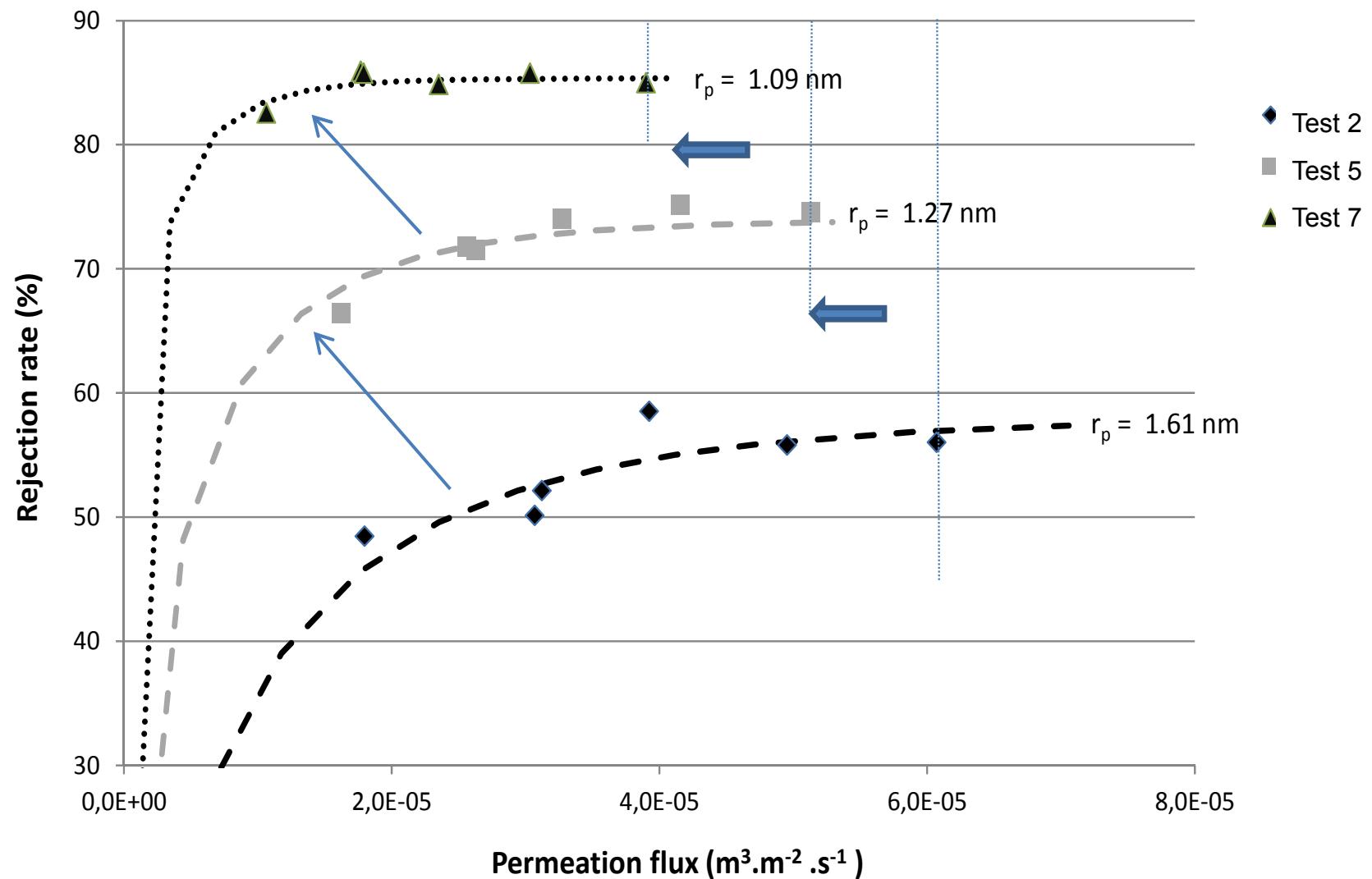
$$R (\%) = \left(1 - \frac{C_{perm}}{C_{ret}} \right) 100$$

**2. Hydraulic performances:
permeability (L_p)**

$$L_p = \frac{n J_v}{\Delta P}$$

N° Test	Solution	R max (%)	Calculated Pore radius (nm)	L_p ($10^{-14} \text{m}^3 \cdot \text{m}^{-2} \text{ memb}$)
	Pure water after conditioning step			6.2
1	L-tyrosine	5		4.7
2	VB12	57	1.61	4.1
3	Lysozyme	85		3.6
4	Lysozyme	93		3.3
5	VB12	75	1.27	3.2
6	Lysozyme	98		3.1
7	VB12	86	1.09	2.4
8	L-tyrosine	6.5		2.3





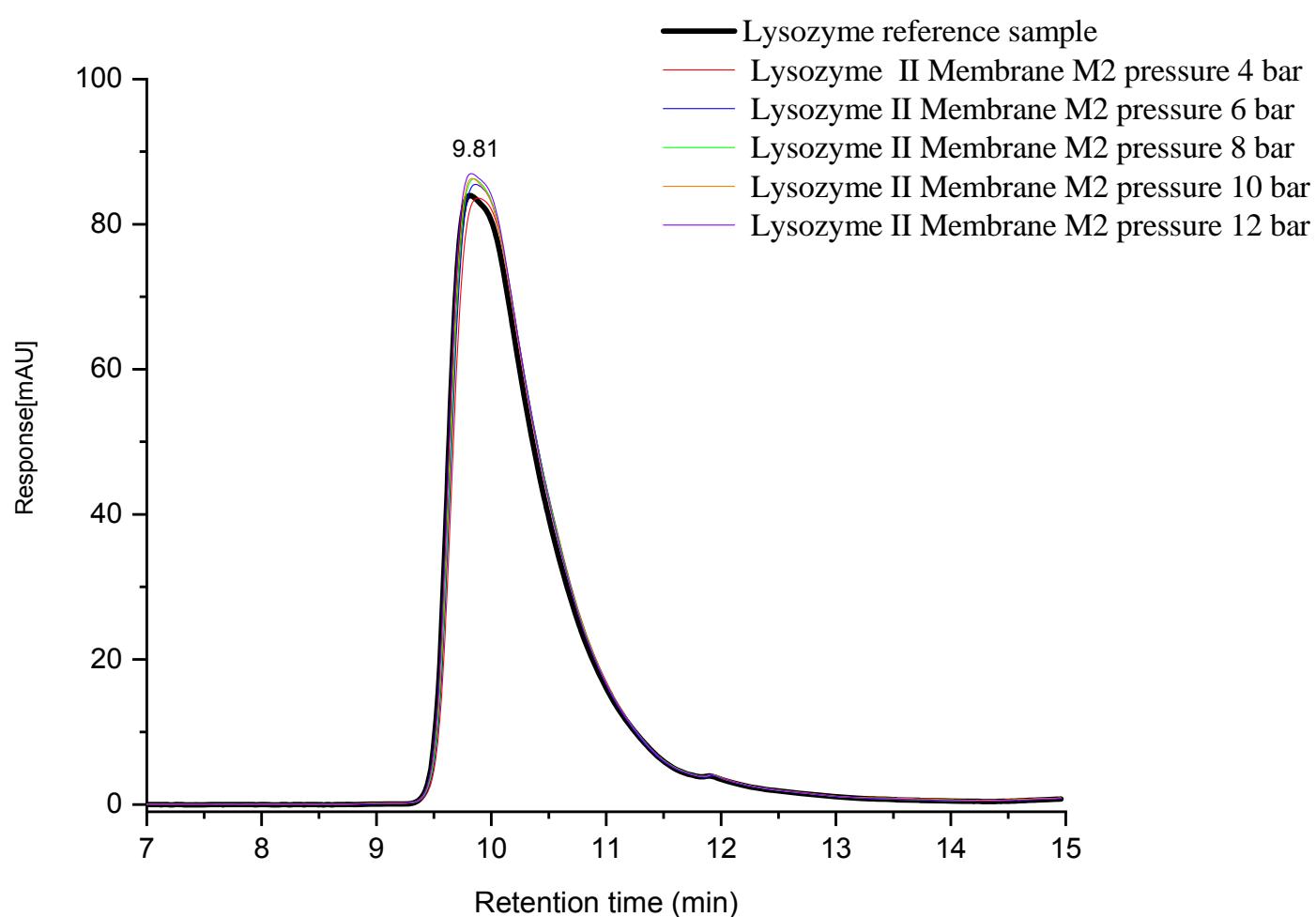


Conclusions from filtration tests

-  membrane hydraulic performances
 -  selectivity properties
 -  lysozyme transmission and hydraulic permeability
  Adsorption of protein  pore clogging
 - Tyrosine filtration after Lysozyme – no variation
 - Tyrosine filtration before lysozyme (clean membrane) - significant effect
- Adsorption of lysozyme in pores increases selectivity and decreases hydraulic performances.***

HPLC analysis of lysozyme

Retentate

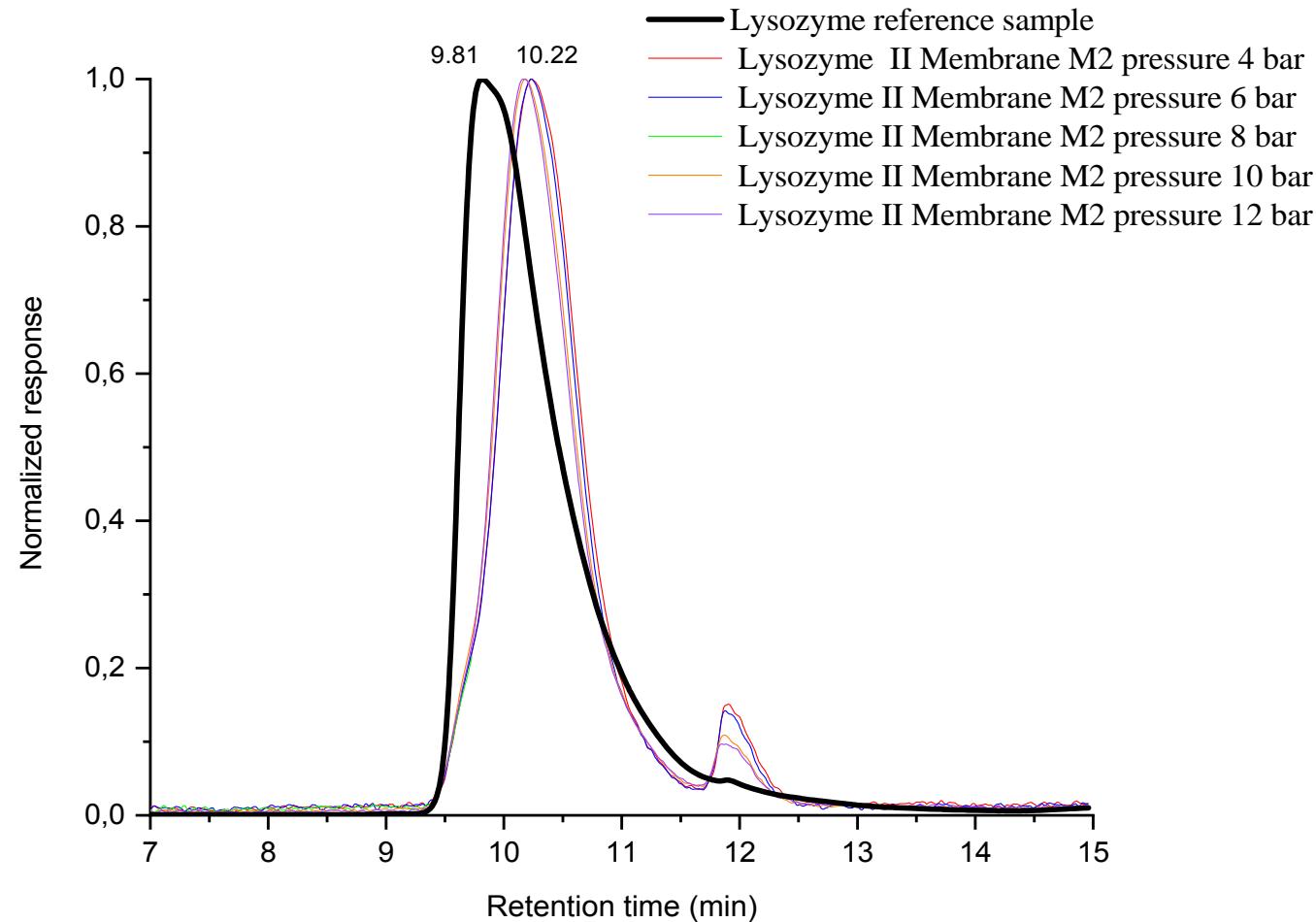


No modification of the lysozyme molecule (no time/ shear stress effect)



HPLC analysis of lysozyme

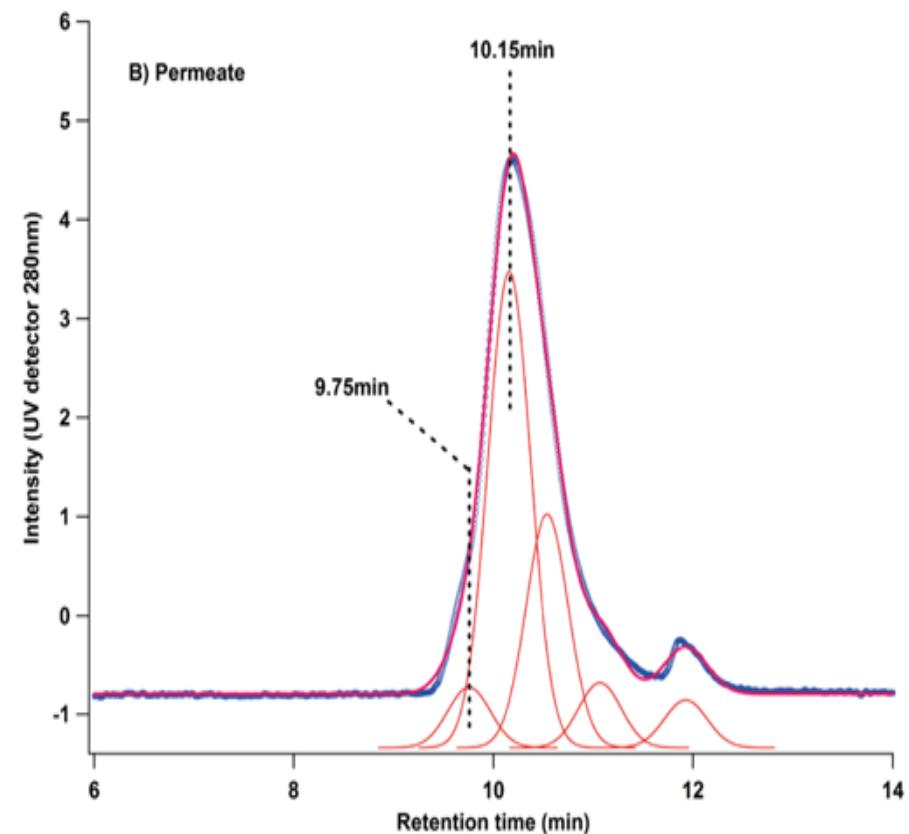
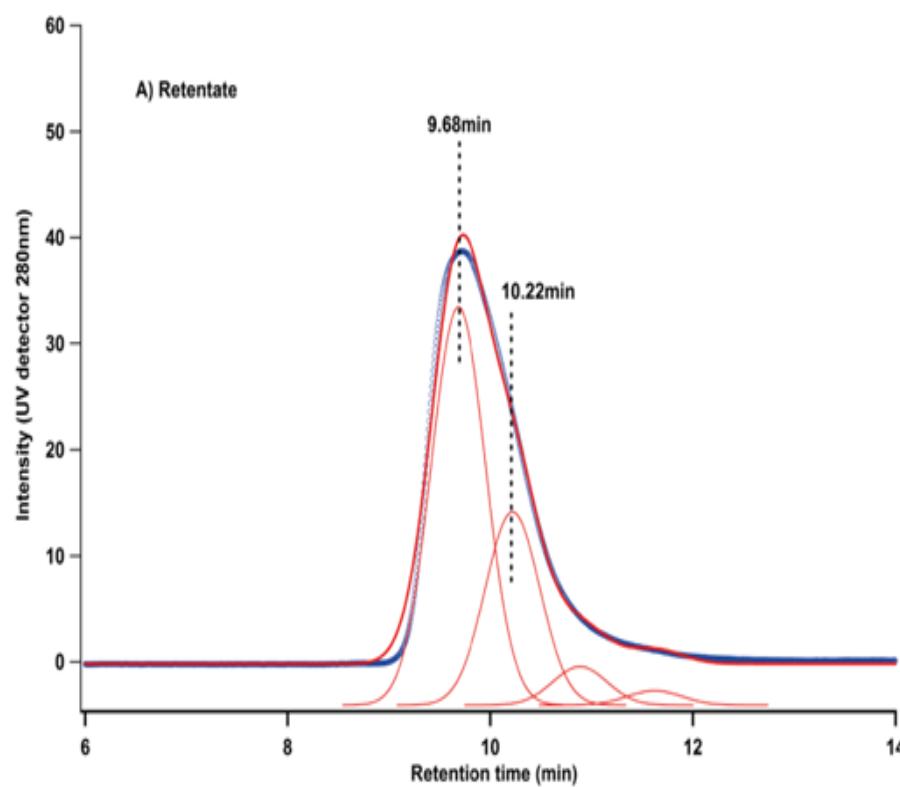
Permeate



Modification of the lysozyme molecule after permeation (denatured)



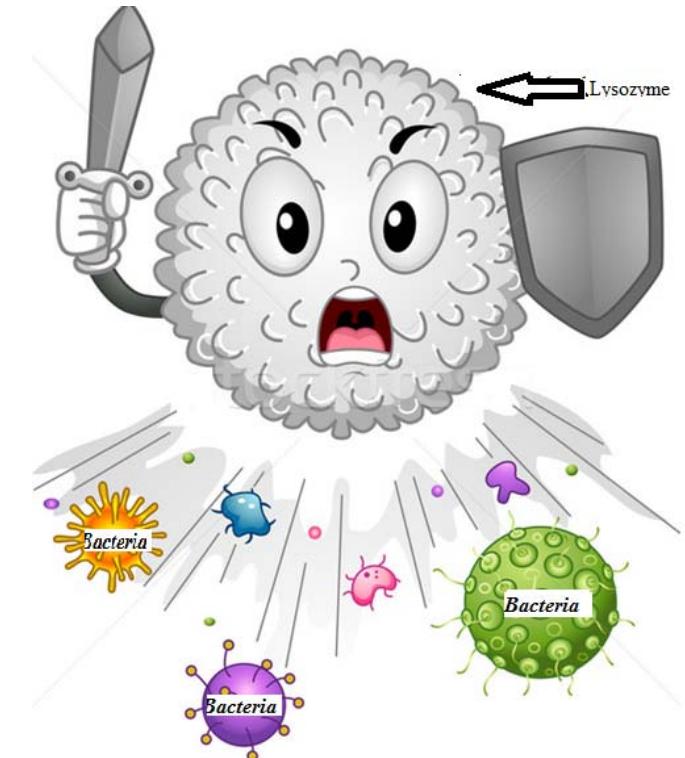
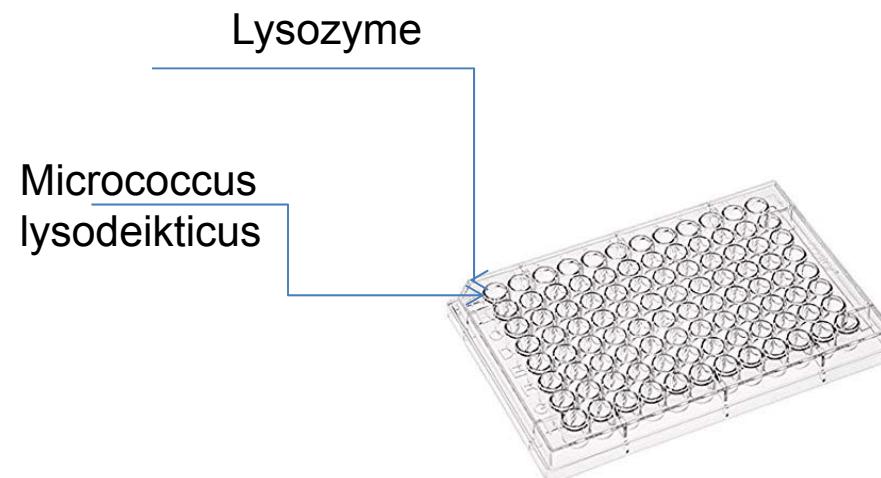
HPLC analysis of lysozyme



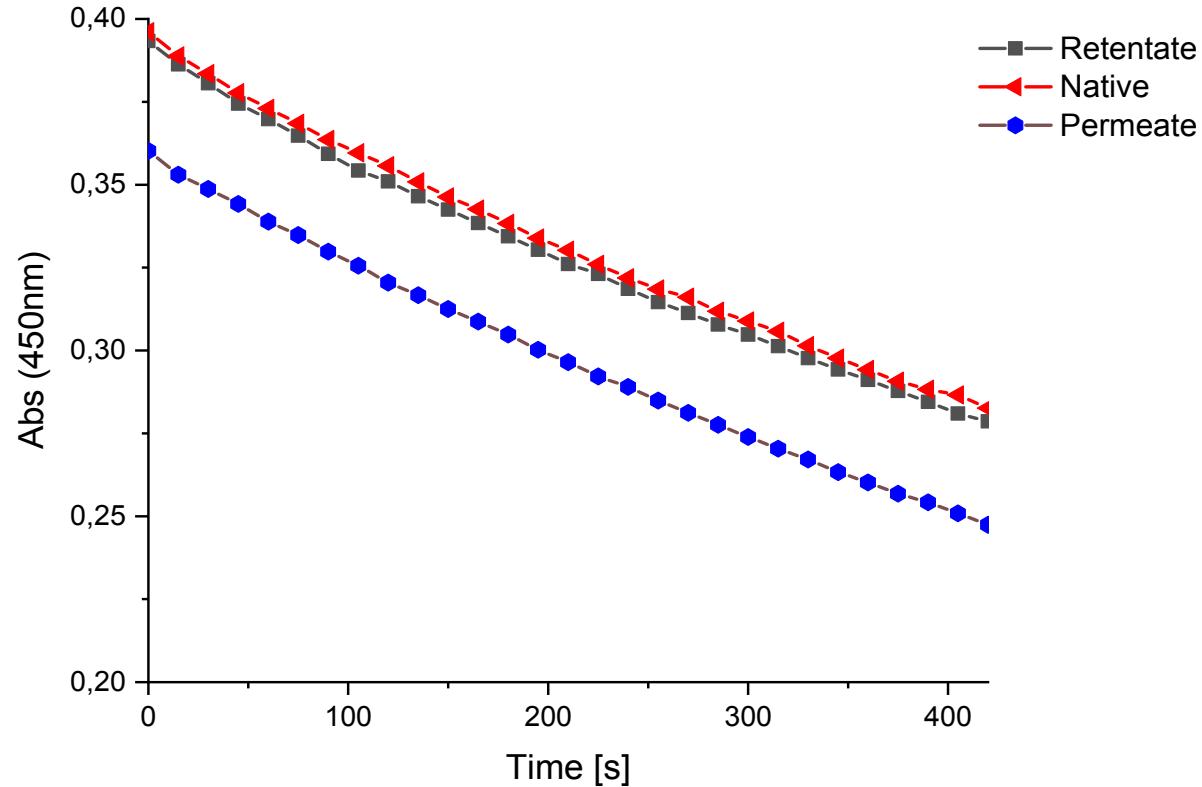
of denatured population of molecules after filtration

Part 2.

Study of biological activity of the filtrated solutions



Lysozyme assay



Sample	Unit/mg	Loss of activity
Native	83 926	-
Retentate	85 528	-
Permeate	68 929	20 %

Retentate shows slightly higher activity than permeate



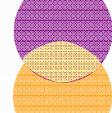
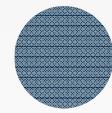
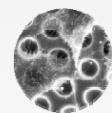
Conclusions/observations



- **Protein adsorption** in larger pores (modification of membrane performances-> Partial fouling)
- **HPLC** : **2 populations of lysozyme with different hydrodynamic radii in retentate and permeate (denaturation)**
- **Loss of conformation not correlated to a huge diminution of bacterial activity**



Institut de Science
des Matériaux de Mulhouse



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Thank you for your attention
Vă mulțumesc pentru atenție





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