Ultrafiltration of protein based solution: Study of membrane fouling

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<table>
<thead>
<tr>
<th>Introduction</th>
<th>Materials and methods</th>
<th>Results and discussion</th>
<th>Conclusions and future work</th>
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</thead>
</table>

**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**
Ultrafiltration
Membrane process

Separate, concentrate, rectify and purify

Water and wastewater treatment

Biopharmaceutical industry

Food and diary industry
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

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)
Membrane characteristics:
- ultrafiltration membrane
- bilayer membrane of TiO$_2$
- asymmetrical pore structure
- cut-off 1 kDa

Membrane performances:

1. Selectivity performances:
   rejection rate (R)

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

2. Hydraulic performances:
   permeability (Lp)

   \[ L_p = \frac{\eta J_v}{\Delta P} \]
<table>
<thead>
<tr>
<th>N° Test</th>
<th>Solution</th>
<th>R max (%)</th>
<th>Calculated Pore radius (nm)</th>
<th>Lp ($10^{-14} \text{m}^3\cdot\text{m}^{-2} \text{memb}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pure water after conditioning step</td>
<td></td>
<td></td>
<td>6.2</td>
</tr>
<tr>
<td>1</td>
<td>L-tyrosine</td>
<td>5</td>
<td></td>
<td>4.7</td>
</tr>
<tr>
<td>2</td>
<td>VB12</td>
<td>57</td>
<td>1.61</td>
<td>4.1</td>
</tr>
<tr>
<td>3</td>
<td>Lysozyme</td>
<td>85</td>
<td></td>
<td>3.6</td>
</tr>
<tr>
<td>4</td>
<td>Lysozyme</td>
<td>93</td>
<td></td>
<td>3.3</td>
</tr>
<tr>
<td>5</td>
<td>VB12</td>
<td>75</td>
<td>1.27</td>
<td>3.2</td>
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<tr>
<td>6</td>
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<td>98</td>
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<td>3.1</td>
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<tr>
<td>7</td>
<td>VB12</td>
<td>86</td>
<td>1.09</td>
<td>2.4</td>
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<tr>
<td>8</td>
<td>L-tyrosine</td>
<td>6.5</td>
<td></td>
<td>2.3</td>
</tr>
</tbody>
</table>

Decrease in lysozyme transmission and in hydraulic permeability $\rightarrow$ adsorption? Pore clogging?
Membrane selectivity increases, hydraulic performances decreases calculated pore radius decreases
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**

![HPLC graph showing retention times for different samples](chart)

- **Lysozyme reference sample**
- **Lysozyme II Membrane M2 pressure 4 bar**
- **Lysozyme II Membrane M2 pressure 6 bar**
- **Lysozyme II Membrane M2 pressure 8 bar**
- **Lysozyme II Membrane M2 pressure 10 bar**
- **Lysozyme II Membrane M2 pressure 12 bar**

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

Permeate

Modification of the lysozyme molecule after permeation (denaturated)
HPLC analysis of lysozyme

↑ of denatured population of molecules after filtration
Part 2.

Study of biological activity of the filtrated solutions

Lysozyme

Micrococcus lysodeikticus
Lysozyme assay

<table>
<thead>
<tr>
<th>Sample</th>
<th>Unit/mg</th>
<th>Loss of activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Native</td>
<td>83 926</td>
<td>-</td>
</tr>
<tr>
<td>Retentate</td>
<td>85 528</td>
<td>-</td>
</tr>
<tr>
<td>Permeate</td>
<td>68 929</td>
<td>20 %</td>
</tr>
</tbody>
</table>

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
Σας ευχαριστώ για την προσοχή σας
Thank you for your attention
Vă mulțumesc pentru atenție
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