ONE-STEP PURIFICATION OF LYSOZYME BY ION EXCHANGE NANOFIBROUS MEMBRANE IN A STIRRED CELL CONTACTOR

1CHUN-TO PENG, 2JUN-YI WU, 3KUEI-HSIANG CHEN, 4YU-KAUNG CHANG

Department of Chemical Engineering/Graduate School of Biochemical Engineering, Ming Chi University of Technology, New Taipei City, Taiwan
E-mail: 4ykchang@mail.mcut.edu.tw

Abstract - Polyacrylonitrile nanofiber membranes (PAN) were fabricated via electrospinning technique. The PAN membrane comprises a polyethylene terephthalate (PET) spunbond fabric as a supporting layer with upper and lower PAN nanofibrous membrane. After NaOH and HCl treatment procedures, the modified membrane is a weak ion exchange membrane (namely AEA-COOH). The AEA-COOH membrane was characterized in terms of fiber diameter, porosity, specific area, pore size, ionic density, and binding capacity. In this study, lysozyme was chosen as a model protein. The adsorption efficiency of the AEA-COOH ion exchange membrane for lysozyme was assessed by the measurements of breakthrough curves in a small stirred cell system (Model Millipore 8010). The capture of lysozyme from diluted chicken egg white (CEW) was carried out with a working volume of 10 mL under the different feed concentrations, flow rates, rotating speeds, and operating pressures. The optimal adsorption conditions were found to be adsorption pH9, 1 in 10 dilution of CEW, average flow rate of 0.1 mL/min, rotating speed of 200 rpm, and initial operating pressure of 0 psi). Furthermore, the elution of lysozyme was also investigated in stirred cell experiments. It was successful in achieving purification of lysozyme in a high yield of 98.2% with a purification factor of 62.9 in a single step.

Index Terms - Chicken egg, electrospinning technique, lysozyme, Polyacrylonitrile nanofiber membranes

I. INTRODUCTION

Electrospinning process has been well known to be a simple and versatile method to produce nanofibrous membranes. Various electrospun nanofibers have been prepared by this method. The nanofibrous membranes with large specific surface area, high porosity, and small interfibrous pore size have been widely applied in tissue engineering, drug delivery, biosensor, ultrafiltration, wastewater treatment, and protein adsorption. Especially, the high specific surface makes the nanofibrous membrane with higher adsorption capacity and faster adsorption rate as compared to conventional microfiberous membranes. Hence, the nanofibrous membranes for use in purification of proteins have been considered as a promising technique because they have better mass transfer performance, higher binding capacity, lower pressure drop, and higher operating flow rate as compared to traditional column processes.

In this work, PAN nanofibrous membranes were prepared by the electrospinning technique and the surface of membrane was hydrolyzed by NaOH to convert nitrile group into carbonyl group. The modified membrane is a weak ion exchange membrane (namely AEA-COOH). The influences of operating parameters (e.g., pH, concentration of CEW, flow rate, rotating speed, and operating pressure) on the adsorption efficiency of the AEA-COOH ion exchange membrane for lysozyme was assessed by the measurements of breakthrough curves in a stirred cell contactor. The purification of lysozyme from diluted chicken egg white (CEW) under the optimal conditions was further carried out in this adsorption process.

II. MATERIALS AND METHODS

A. Materials
The fresh chicken eggs were purchased from a local supermarket (Taipei, Taiwan). Homogenizer was obtained from Hsiang-Tai Machinery Industry Co., Ltd., (Taipei, Taiwan). Polyacrylonitrile (PAN) yarn (Mw 120,000 g/mol, containing 93% acrylonitrile and 7% vinylacetate) was purchased from Fortune Industries Inc. (Tao-Yuan, Taiwan). Polyethyleneterephthalate (PET) spunbond fabric (basis weight 15g/m², thickness 85 µm) was supplied from Freudenberg Far Eastern Spunweb Co. Ltd. (Taipei, Taiwan). All other chemicals and solvents were used without further purification and all electrospinning processes were conducted at room temperature. Electrospinning device was purchased from Jyi Goang Enterprise Co., Ltd. (Taipei, Taiwan). A UV-vis spectrophotometer (Model Ultrospec 3100 pro, Amershams Biosciences, Uppsala, Sweden) was used to measure the contaminating protein concentration and lysozyme activity. The stirred cell contactor (Model Millipore 8010) was used for the experiments.

B. Preparation of PAN nanofibrous membrane
The preparation of PAN nanofibrous membranes was described previously by Chiu et al.

C. Preparation of weak ion exchange membrane
The PAN nanofibrous membrane was cut into 4.9 cm² (i.d. 2.5 cm), placed in 3 M NaOH at 85°C for 20-25 min. After alkaline treatment, the membrane was successively washed H₂O to remove the excess NaOH. The membrane was then treated with 0.1 M HCl, followed by drying in an oven at 60°C before use.

Proceedings of 110th The IRES International Conference, Osaka, Japan, 8th-9th April, 2018

35
**D. Total protein and lysozyme activity assay**

The total protein concentration was measured by the Bradford method using lysozyme as the standard. The activity of lysozyme was measured by lysis of 0.25 mg/mL M. lysodeikticus cells in 100 mM sodium phosphate (pH 6.24) at 25°C. One unit (U) of lysozyme activity was defined as the decrease of OD295 by 0.001 per minute.

**E. Choice of adsorption pH**

The crude CEW was separated from fresh eggs and homogenized in an ice bath at 2,000 rpm for 30 min. The homogenized CEW was then diluted to 10% (v/v) with 20 mM relevant buffer (i.e., sodium acetate buffer, pH 4–5; sodium phosphate buffer, pH 6–8; glycine-NaOH, pH 9–10; sodium carbonate buffer, pH 11–12). The balance in dilution of crude CEW (general protein 4.31 mg/mL, lysozyme activity 9,550 U/mL, specific activity 2,214 U/mg) at the relevant pH 4–12 was used as a lysozyme source. For batch studies, an 1 in 10 dilution of CEW was made up in 5 mL of 20 mM relevant buffer (pH 4–12) in separate flasks. A piece of membrane (PAN-PET-PAN) was added to each flask. The flasks were sealed and placed in a shaker at 25°C, 100 rpm for 3 h. After this period had elapsed, sample (1 mL) of supernatants collected in each flask was assayed for protein concentration and lysozyme activity. The adsorption capacity of the membrane was determined by measuring the initial and final activity of lysozyme and was described as the amount of lysozyme (U) per gram of the membrane. The amount of adsorbed lysozyme on the membrane (U/g-M) was calculated according to the following equation:

\[ q = \frac{v(A_i - A)}{w} \]  

where \( A_i \) and \( A \) (U/mL solution) are the initial and final activity of lysozyme in the liquid phase, respectively. \( q \) (U/g-M) is the final activity of lysozyme in the membrane solid phase. \( v \) is the volume of liquid phase (mL) and \( w \) is the weight of membrane (g).

**F. Dynamic adsorption process by stirred cell contactor**

The dynamic adsorption rate for lysozyme onto the membrane are measured with a stirred ultrafiltration cell (Amicon stirred cell Model 8010, membrane diameter is 25 mm; effective filtration area is 4.1 cm², working volume 10 mL). The maximum operation pressure of the stirred cell was 75 psi (5.3 kg/cm²) and a stirrer assembly mounted inside the cell has a diameter of 17 mm. The fabricated membrane is placed in the cell. The applied pressure of stirred cell was controlled by nitrogen gas, and the rotating speed was maintained at a fixed value. The CEW solution in the cell is stirred and pressed by N₂ gas to go through the membrane. The cell was allowed to equilibrate for 30 min before any experiment was started. All the experiments were carried out with 10 mL of feed CEW solution. The CEW concentrations at the inlet and outlet are measured to determine the dynamic binding capability of membrane (U/g-M).

The average flow rate and flux of CEW is obtained by measuring the volume of CEW passed through the membrane at a given time interval. All the feed CEW solutions are prepared at a range of protein concentration and lysozyme activity, respectively. The permeate was collected in every increment of 1 mL.

**G. Purification of lysozyme from chicken egg white in a stirred cell contactor**

A 10 mL dilute chicken egg white solution (10%, pH 9) prepared from a fresh egg white was pumped through the membrane at average flow rate of 3.2 mL/min, rotating speed of 200 rpm, and operating pressure of 0 psi. After washing stage, the non-adsorbed components in CEW were removed from the membrane using the adsorption buffer. Elution was carried out using two-step scheme (0.6 and 1.0 M NaCl, pH 9). The effluents for each stage were collected to determine the general protein concentration and assay the lysozyme activity.

**III. RESULTS AND DISCUSSION**

**A. Dynamic adsorption process by stirred cell contactor**

Purification of lysozyme from CEW by a stirred cell contactor is a rather complex process which is influenced by a number of parameters relating to the dilution of CEW, liquid flow rate, rotating speed, and operating pressure. To find suitable operating conditions for the purification of lysozyme by stirrer cell contactor, some operating parameters were varied as shown in Table 1.

<table>
<thead>
<tr>
<th>Variations of operating parameters in stirred cell processes</th>
<th>Operating conditions</th>
<th>Value/Range (Constant parameters)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dilution of CEW</td>
<td>1 in 4, 1 in 14</td>
<td>1 in 4.31 in 14 (10 rpm and 0 psi)</td>
</tr>
<tr>
<td>Rotating speed (rpm)</td>
<td>0-400</td>
<td>600 rpm, and 0 psi</td>
</tr>
<tr>
<td>Initial liquid flow rate (mL/min)</td>
<td>5-100</td>
<td>10 mL, 10 mL/min, and 0 psi</td>
</tr>
<tr>
<td>Operating pressure (psi)</td>
<td>0</td>
<td>0.6 and 1.0 M NaCl(PRE)</td>
</tr>
</tbody>
</table>

The adsorption capacity for lysozyme from CEW was firstly investigated in various dilutions of CEW between 1 in 4 and 1 in 14. Comparison of adsorption capacity for general protein and lysozyme, the percentage of adsorption for lysozyme is much higher than that for general protein. Therefore, the adsorption behavior for lysozyme onto AEA-COOH membrane was much more selective than that for general protein as shown in Fig. 1. At higher concentration of CEW, it is less favorable to the adsorption of lysozyme. The maximum adsorption (95.3%) for lysozyme was obtained in 1 in 10 dilution. The purification results showed that lysozyme could be directly recovered by 1.0 M NaCl from CEW with a purification factor of 59.0 and yield of 94.7% in a single step. Hence, the optimal dilution factor for CEW was 10.

The adsorption of lysozyme from CEW was further investigated at variable liquid flow rates regulated with
a flowing valve. The processing time decreased with increasing the liquid flow rate. On the contrary, the purification factor increased with increasing the liquid flow rate. The results are shown in Fig. 2. As initial liquid flow rate was increased from 3 to 20 mL/min, the adsorption for general protein increased from 1.7 to 4.7%. However, the lysozyme adsorption decreased from 98.2 to 95.3%. This would result in the increase of purification factor from 21.5 to 59.0. Hence, it was clearly evident that the maximal purification factor for purification of lysozyme was observed at an initial flow rate of 20 mL/min (average flow rate of 3.2 ml/min).

As expected, the rotating speed does not significantly affected the adsorption capacity and processing time under the process conditions as shown in Fig. 3. However, at rotating speed of 200 rpm, the maximal adsorption (%), recovery yield (%), and purification factor would be observed at 98.9%, 99.5%, and 64.5, respectively. Hence, the optimal rotating speed was chosen at 200 rpm.

The adsorption of lysozyme from CEW was finally investigated at various operating pressures in the range of 0-60 psi. The results are shown in Fig. 4. The percentage of adsorption for lysozyme increased from 64.1 to 98.9% with decreasing the operating pressure from 60 to 0 psi. However, the adsorption for general protein increased from 1.6 to 3.0%. The recovery yield increased from 63.0% to 99.5% and the purification factor increased from 25.6 to 64.5, respectively with decreasing the operating pressure from 60 to 0 psi. Hence, lower operating pressure would be more suitable for use in the purification of lysozyme. As investigations reported above, one set of operating parameters for the purification of lysozyme form CEW was 1 in 10 dilution of CEW, rotating speed of 200 rpm, initial liquid flow rate of 20 mL/min (average flow rate of 3.2 ml/min), and initial operating pressure of 0 psi.

As described above, appropriate operating conditions for the purification of lysozyme has been established for experiments conducted with clarified CEW and stirred cell reactor (Model 8010). The volume of CEW loaded onto the reactor (10 mL) and AEA-COOH membrane (4.1 cm²) was used in this study. The results of this experiment were shown in Fig. 5 and Table 2. The level of general proteins and lysozyme activity in the effluent increased to 100% and 1.2% of the inlet value, respectively.
B. Purification of lysozyme from CEW in stirred cell contactor

As described above, appropriate operating conditions for the purification of lysozyme have been established for experiments conducted with clarified CEW and stirred cell reactor (Model 8010). The volume of CEW loaded onto the reactor (10 mL) and AEA-COOH membrane (4.1 cm²) was used in this study. The results of this experiment were shown in Fig. 5 and Table 2. The level of general proteins and lysozyme activity in the effluent increased to 100% and 1.2% of the inlet value, respectively.

Two-step elution protocol used was devised in the experiments. The first elution step (0.6 M NaCl, pH 9.0) can elute 97.2 and 99.4% of bound general protein and lysozyme, respectively. The lysozyme was recovered with a yield of 98.2% and a purification factor (PF) of 63. The second elution step (1.0 M NaCl, pH 9.0) eluted other remaining adsorbed proteins and lysozyme.

Table 2. Purification of lysozyme from CEW by AEA-COOH ion exchange nanofibrous membrane

<table>
<thead>
<tr>
<th>Purification step</th>
<th>Loading volume</th>
<th>Total protein</th>
<th>Total proteins</th>
<th>Specific activity (U/mg)</th>
<th>Lysozyme yield (%)</th>
<th>PF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial treatment</td>
<td>10</td>
<td>64.27</td>
<td>98.08</td>
<td>2.89</td>
<td>100.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Flow through</td>
<td>15</td>
<td>47.81</td>
<td>1.208</td>
<td>96.25</td>
<td>1.14</td>
<td>0.91</td>
</tr>
<tr>
<td>Wash</td>
<td>15</td>
<td>1.67</td>
<td>6.37</td>
<td>7.1</td>
<td>1.68</td>
<td>0.97</td>
</tr>
<tr>
<td>Wash with buffer</td>
<td>15</td>
<td>5.16</td>
<td>94.18</td>
<td>2.16</td>
<td>99.20</td>
<td>63</td>
</tr>
<tr>
<td>1.0 M NaCl (pH 4.2)</td>
<td>15</td>
<td>4.76</td>
<td>109.10</td>
<td>2.39</td>
<td>99.94</td>
<td>64</td>
</tr>
<tr>
<td>4.0 M NaCl (pH 2.0)</td>
<td>15</td>
<td>4.55</td>
<td>365</td>
<td>2.99</td>
<td>89.90</td>
<td>1.61</td>
</tr>
</tbody>
</table>

Fig. 5. Purification of lysozyme from clarified CEW by using AEA-BrA nanofibrous membrane

CONCLUSION

On the basis of these results, two-step elution scheme was very effective for the purification of lysozyme from clarified CEW. The results demonstrated that the applicability of nanofibrous membrane technique for the purification of lysozyme from CEW in stirred cell contactor would be possible. The recovery yield of purification process was higher than other conventional membrane purification processes.

ACKNOWLEDGEMENT

YKC gratefully acknowledges the financial support provided by the National Science Council, Taiwan (NSC 102-2221-E-131-032).

REFERENCES


★★★★