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Antifouling PES Membranes Using Bio-Catalyzed Surface Modification

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ABSTRACT

An effective antifouling modification of poly(ethersulfone) (PES) membranes using ortho-aminophenol modifier and laccase from *Trametes versicolor* biocatalyst under mild conditions is presented in this paper. The modified PES membranes are evaluated using many techniques including total color change, pure water flux, and protein repellence and related to the gravimetric grafting yield. Membrane surfaces were imaged by SEM to illustrate the formed modifying poly(2-aminophenol) layer(s) at different modification conditions. The clean water flux of the most modified membranes was increased up to 15.4 % relative to the unmodified membrane and reduction in protein adsorption up to 81.27% was obtained.

1. INTRODUCTION

In polymerization of aminophenol, the relative position of amino and hydroxyl group is important. The reported electrochemical properties of the three positional isomers (ortho, meta, and para) are strongly different [Salavagione2004, Kong 2011]. The electrochemical oxidation of 4-Aminophenol (4-AP)on a mercury electrode in aqueous medium indicated that the electrooxidation of 4-APproduces4-benzoquinone through two sequence steps [Chandrashekar 2011], which has been assisted by other researchers as a result of their effort to form polymer films on other different electrode materials [Schwarz 2003, Wang 2013]. Also, the ability of chemical oxidativly polymerized 4-AP and its nanocompound on growth inhibition of both *Staphylococcus aureus* (gram +ve) and *Escherichia coli* (gram-ve) bacterial strains at different levels has been reported [Thenmozhi 2011].

The ortho-aminophenol (2-AP) has a high electronic density in the para positions with respect to the $–NH_2$ group. Therefore, dimers could be formed through attack of the cation radical at that position. The dimer of 2-AP has the higher electronic density in the para

position with respect to the –OH group, allowing closing of the phenoxazine ring. The resulting polymer will have aladder type structure built in by phenozaxine units [Jackowska 1993 and Scherf 1999]. But the products of 2-AP oxidation on a roughened silver electrode in alkaline and neutral media was identified as a linear dimer formed by N–N coupling; 2,2′-dihydroxyazobenzene [Pałys 2010].

Poly(arysulfone) membranes such as Poly(ethersulfone) show a high binding affinity for different molecules such as proteins and microorganisms, which causes severe fouling of membranes during different processes such as desalination. To diminish such fouling, various methods have been proposed to alter the surface properties of poly(arylsulfone) membranes, and to reduce adsorption of different foulants [Rana 2011, Nady 2011]. In our previous researches, we investigate the modification of poly(ethersulfone) (PES) membranes using Laccase from *Trametes versicolor* that able to oxidize phenolic compounds [Mayer 2002,Riva 2006,Witayakran 2009] to yield reactive radicals that can covalently bind to each other (polymerization) and/or onto PES membrane (bio-grafting), mainly via their OH-groups [Nady 2011 and 2012]. Furthermore, this laccase-catalyzed modification has been used to modify the PES membrane using 4-aminophenol isomer and significant reduction in protein adsorption was obtained [Nady 2014].

In the current work, we use 2-AP isomeras modifier (substrate for laccase or monomer) and enzyme laccase biocatalysis to modify the PES membrane surfaces. Two different concentration of modifiers were used to modify the PES membranes at various modification times using 0.5 $U \cdot ml^{-1}$laccase in 0.1 M sodium acetate buffer pH 5.5. A variety of techniques was used to characterize both the blank and modified membranes including e.g. quantified total color changes, gravimetrically determined grafting yields, pure water flux, protein repellence (reduce protein adsorption can be considered as a first step to reduce biofouling), and flux reduction resulted from protein adsorption.SEM was used to further characterize the modified membranes. The combination of these data allows an outlook on laccase-catalyzed modification of membranes using 2-aminophenol modifier.

2. EXPERIMENTAL

2.1 Chemicals.

2-Aminophenol (2-AP, ≥99.5%) was obtained from Sigma-Aldrich. Catechol (>98%) was provided from Oxford Laboratory Reagent (India). Sodium acetate (anhydrous, ≥99%) was purchased from Polskie OdczynnikiChemiczne S.A., (Poland). Acetic acid (99.9%) was supplied from Laboratory Chemicals (Egypt). Flat sheet commercial poly(ethersulfone) membrane: Sartorius (symmetric, 0.2 µm pore size, 50 mm diameter, 150 µm thickness, water flow rate > 28 $ml \cdot min^{-1} \cdot cm^{-2}$ at ΔP = 1 bar). Laccase from *Trametes versicolor* (>10.4 $U \cdot mg^{-1}$, Fluka). All chemicals were used as received. Deionized water was used in all experiments. All used solutions are prepared freshly before using.

2.2 Laccase Assay.

The laccase activity was determined with catechol as substrate. The assay mixture contained 0.33 ml of 10 mM catechol, 2.67 ml of 0.1 M sodium acetate buffer (pH 5), with 0.025 $U \cdot ml^{-1}$laccase. Oxidation of catechol is monitored by following the increase in absorbance at 400 nm (ε = 26,000 $M^{-1} \cdot cm^{-1}$) [Singh 2007], with a reaction time of 20 min. One unit of laccase activity is defined as the amount of enzyme required to oxidize 1 µmol of catechol per min at 25±1 °C.

2.3 Membrane Modification.

The flat sheet membranes were immersed in 40 ml sodium acetate buffer containing different concentrations of 2-aminophenol and enzyme laccase. Air was supplied as O_2 source and was used for gentle continuous mixing to ensure a homogenous reaction medium. After a specific modification time (30, 60, or 120 min), the membranes were removed from the reaction medium and washed by strong spray flushing with deionized water, followed by three times dipping and shacking in freshly boiled deionized water (~ 100 ºC). The modified membranes were dried in glass dishes placed in desiccators supplied with self-indicating blue silica gel for 48 h before evaluation.

2.4 Color Measurements.

The CIELAB coordinates for the modified membranes were measured with an X-Rite (SP62 Sphere Spectrophotometer, CIE L*a*b*, and ΔE* at D 65/10º). The color values L* (lightness), a* (red-green axes), b* (yellow-blue axes), and E* (the degree of total color change) were determined relative to the unmodified membrane as standard (compare mode; ΔL*, Δa*, etc). Aperture size was 8 mm diameter. Three readings were taken from three different places on each sample and the average value was calculated.The membranes were washed by filtration with at least 200 ml deionized water and then dried for 48 h before the actual color change was measured.

2.5 Pure Water Flux.

A dead-end stirred filtration cell (Millipore, Model 8050, active transport area 13.4 cm^2) was used to measure pure water flux of unmodified and modified membranes at a constant trans-membrane pressure of 1 bar at 24±1 ºC and 200 rpm. The pure water flux was calculated with Equation 1, in which J_w = water flux ($m^3 \cdot m^{-2} \cdot s^{-1}$), Q is the volume of permeate collected (m^3), Δt is the sampling time (s), and A is the membrane area (m^2).

$$J_w = \frac{Q}{\Delta t \cdot A} \qquad (1)$$

2.6 Grafting Yield.

The amount of material grafted onto the membrane surface was calculated from the difference in weight of the membrane, before and after grafting process relative to the membrane unit area. Before grafting, all the membranes were kept for 48 h in glass-

covered dishes in desiccators supplied with self-indicating blue silica gel to remove any moisture.To remove any enclosed or loosely bound material, the weight of the membrane after grafting was measured after washing the membrane by filtration of at least 300 ml deionized water.

2.7 BSA Adsorption.

BSA was used as a model compound to evaluate protein adsorption on unmodified and modified membranes as described in our previous research [Nady 2012]. Briefly,the membranes were immersed in 50 ml BSA solution ($1g \cdot l^{-1}$ BSA solution at pH 7 was prepared using 0.1 M sodium acetate buffer) and gently shaken (200 rpm) at 25 °C for 24 h. BSA concentration in the solution was measured using a UV-Vis spectrometer (280 nm), and from this the adsorbed amount was calculated.

2.8 Scanning Electron Microscope (SEM).

Unmodified and modified membranes were imaged using a Scanning Electron Microscope (JeolJsm 6360LA, Japan). The membrane samples were cut using a very sharp shaving blade and were then coated with Au, and imaged at a voltage of 30 KV, and a resolution of 1280 × 960 pixels.

2.9 Mechanical Properties.

Samples were cut in a dog-bone-like shape. The total length of each sample was 37 mm, the gauge length of the samples was about 16 mm; the width was 13 mm at the top and 7.2 mm (narrowest) at the middle of the sample, to force a fracture in the middle of the sample. Tensile testing of the films was performed with the Texture Analyzer T2 (Stable Micro Systems, Ltd., Surrey, United Kingdom), at a constant crosshead speed of 6 mm/min until breaking. Stress–strain curves were calculated from load–elongation curves measured for 2 samples from each film. The tensile strength was calculated from the stress–strain curves.

3. RESULTS AND DISCUSSION

2-Aminophenol (2-AP) was used to modify PES membranes using laccase biocatalyst. The laccase used to catalyze creation of the free radicalform of the modifier 2-AP requiredto graft onto the PES membrane. The formed free radicals can react with each other to form homopolymer (Nady 2011). For that, the samples were analyzed after extensive washing using boiled water (100°C) until clear washing water is obtained as measured using spectrophotometer. The total color change (ΔE^*) as function of grafting yield are illustrated in Fig. 1.

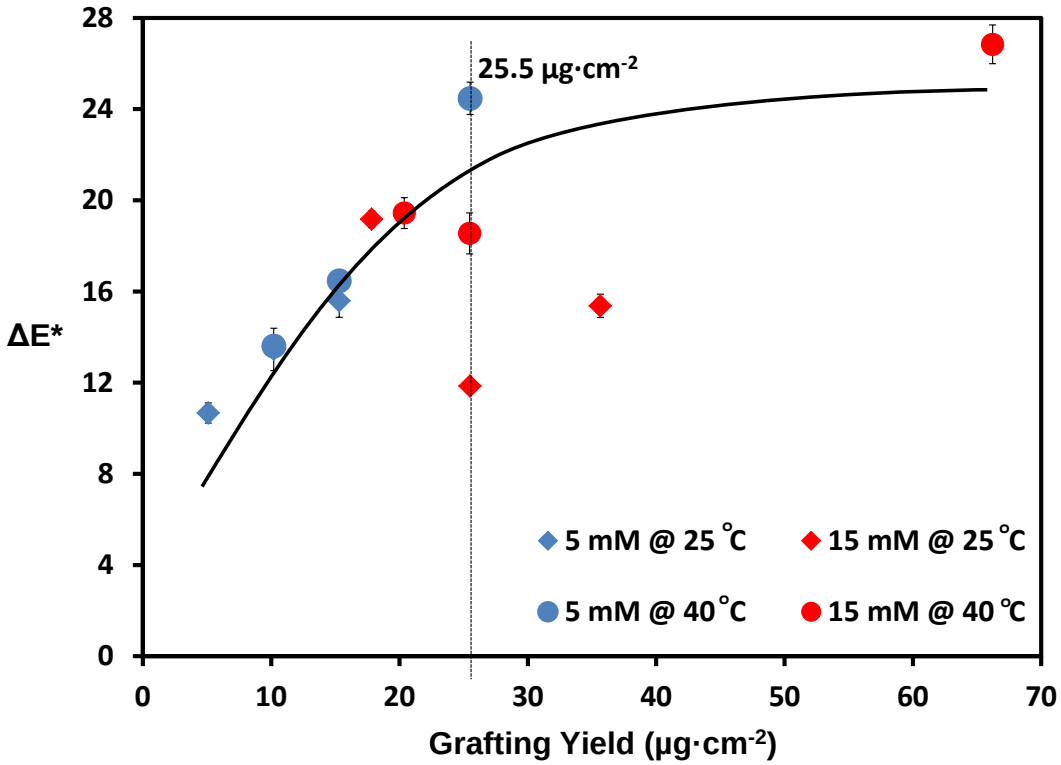

Fig. 1. Total color change (ΔE*) as a function of grafting yield; the reference reaction condition is 0.5 U·ml^{-1}laccase, pH 5.5, and 0.1 M sodium acetate buffer. Two concentrations of 2-aminophenol modifier are used (5 mM; blue and 15 mM; red). The membranes are modified at two modification temperatures (25ºC; diamond and 40ºC; circular) with different modification times (30, 60, and 120 min). Guide lines for general performance (solid black line).

Different modification conditions are used: two concentrations of 4-AP (5 and 15 mM), two modification temperatures (25 ºC and 40 ºC), and different modification times (30, 60, and 120 min) using 0.5 U·ml^{-1} laccase and 0.1 M sodium acetate buffer; pH 5.5. As noticed in Fig. 1, the total change of color increased gradually with increasing grafting yield up to 66.21 μg·cm^{-2} (ΔE* = 26.2 at 15 mM 2-AP, 120 min modification time, and 40 ºC reaction temperature). The lower coloration at high concentration and longer modification time for modification at 25°C may be attributed to lower enzyme activity and/or poor aqueous solubility of the 2-AP at 25°C reaction temperature. Similar to previous study using 4-aminophenol isomer, three different color changes at the same grating yield was determined (about 25.5 μg·cm^{-2}grafting yield at 40 ºC: 5 and 15 mM 2-AP with 120 and 60min modification times, respectively; At 25 ºC: 15 mM 2-AP at 120 min modification

time). However, the case here with 2-AP differ than the case of 4-AP in protein repellence [Nady2014]; It seems the three modifications conditions produce the same grafting yield and the same shape and/or structure because they show almost the same protein repellence (see the following section).Also, at grafting yield equal or greater than 25 $\mu g \cdot cm^{-2}$, there is no clear relation between the grafting yield and total color change (ΔE^*). This could be explained that the increasing in the added modifier accompanied with difference in color saturation (not measured here) instead of total change of color that most probably due to formation of more layers instead of increase the grafting density (increase the length of the grafted oligomers rather than increase the number of grafted oligomer chains). This explanation in full agreement with our conclusions from previous study using 4-hydroxybenzoic acid [Nady 2012], modifier with two legs tends to grow in one dimension with grow the grafted chains on the surface. The clean water flux of the most modified membranes increases up to 15.4% relative to the unmodified membrane (see Fig.2).

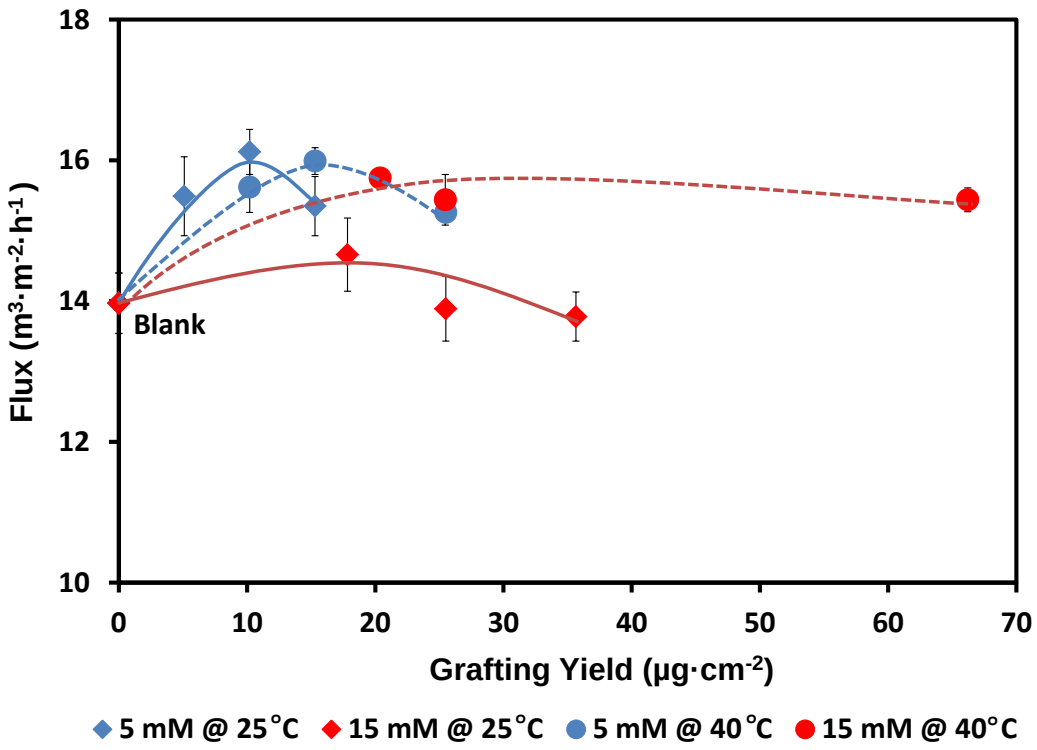

Fig. 2. Clean water flux as a function of grafting yield; the reference reaction condition is 0.5 $U \cdot ml^{-1}$laccase, pH 5.5, and 0.1 M sodium acetate buffer. 2-aminophenol (5 mM; blue and 15 mM; red) modifier is used at two reaction temperature (25ºC; diamond and 40ºC; circular) with different modification times (30, 60, and 120 min).

This is could be related to formation of homogenous layer without plugging the pores as show in SEM in the following section. The highest clean water flux reduction is not at the highest grafting yield but it was found to be 1.4 % for membrane modified using 15 mM 2-AP in combination with 120 min modification time and 25°C reaction temperature. Also, we can observe the same trend for each studied condition; first increase the pure water flux and then decreased with increases the grafting yield. This trend may be attributed to decrease the number of open (free) groups that have ability to bond the water molecules and facilitate the water flux. So, the low grafting yield may show better flux and better protein repellence as illustrated in the following sections.

The overall performance proved that the adsorbed amount of bovine serum albumin (BSA, see Fig. 3) decreases with modification but there is no direct relationship between the grafting yield and the extent of protein repellence of the modified membranes.

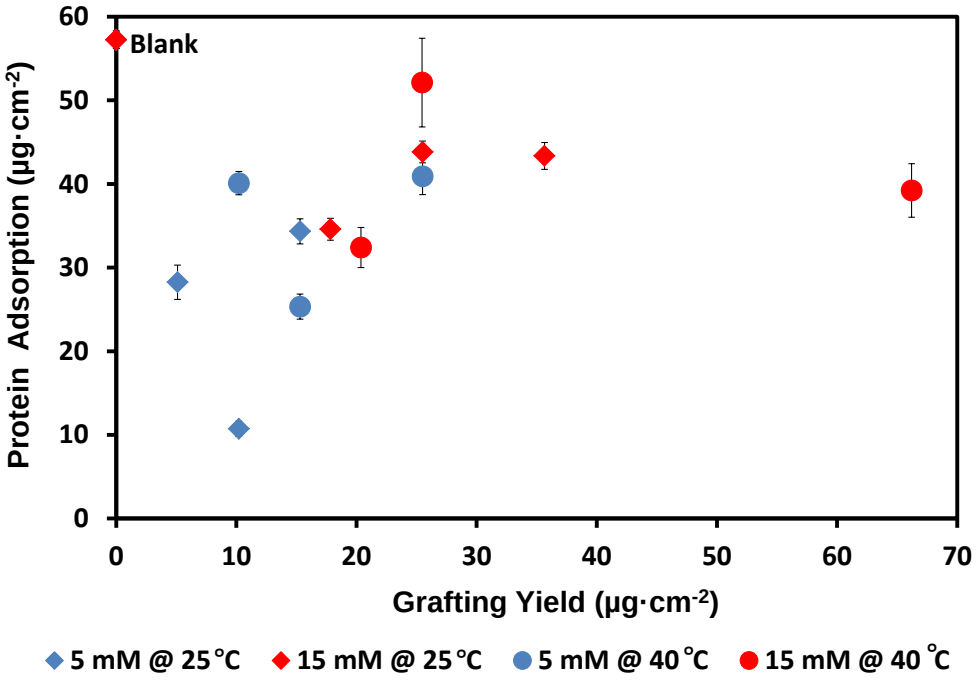

Fig. 3. Protein adsorption as a function of grafting yield; the reference reaction condition is 0.5 U·ml^{-1}laccase, pH 5.5, and 0.1 M sodium acetate buffer. 2-aminophenol (5 mM; blue and 15 mM; red) modifier is used at two reaction temperature (25ºC; diamond and 40ºC; circular) with different modification times (30, 60, and 120 min).

However, it well observed that the best protein repellence obtained with modified membranes using low modifier concentration (up to 81.27 % reduction in protein adsorption was obtained using 5 mM 2-AP and 60 min modification at 25 ºC).We can see

full agreement with trend of the individual studied modification conditions (temperature, modifier concentration, etc.) in pure water flux and protein repellence. Increase the grafting yield, firstly lead to increase the flux that accompany with improvement in protein repellence, then both of pure water flux and protein repellence slightly decreases with further increasing in the grafting yield.
The flux reduction due to irreversible protein adsorption (unremoved protein after back and forward washing) is much lower than the flux reduction of the unmodified membrane as shown in Fig.4.

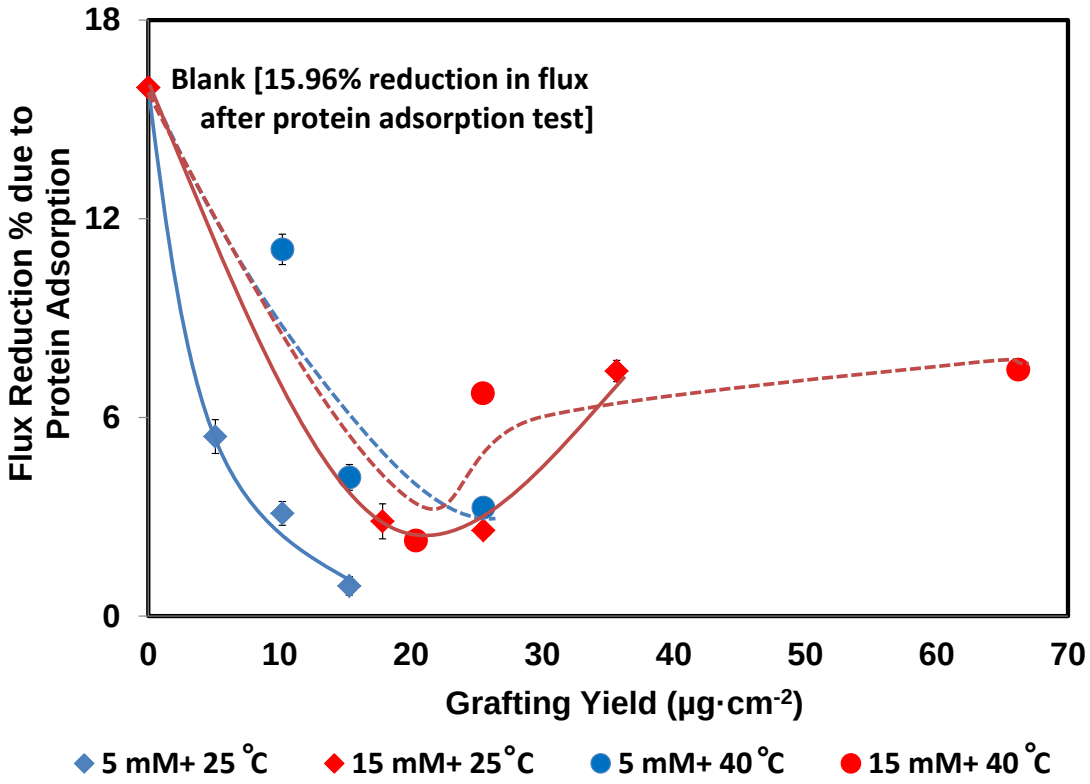

Fig. 4. Protein adsorption as a function of grafting yield; the reference reaction condition is 0.5 U·ml^{-1}laccase, pH 5.5, and 0.1 M sodium acetate buffer. 4-aminophenol (5 mM; blue and 15 mM; red) modifier is used at two reaction temperature (25 ºC; diamond and 40 ºC; circular) with different modification times (30, 60, and 120 min).

The flux of unmodified membrane reduced by 15.96 % of its original flux, whereas all the modified membranes showed residual fluxes much better than the residual flux of unmodified membrane. For example, the modified membrane that showed the best protein repellence lost 3.1% of its original flux (after modification). So, the flux of this modified membrane after both modification and protein adsorption is still higher than the flux of the unmodified membrane before protein adsorption by %11.81 and by % 33.4 than the flux of

the unmodified membrane after (irreversible) protein adsorption (*i.e.*,fluxes due to irreversible protein adsorption are 11.74 and 15.62 $m^3 \cdot m^{-2} \cdot hr^{-1}$ for unmodified and modified membranes, respectively). Also, the final flux of the modified membrane that acquired the highest grafting yield (66.21 $\mu g \cdot cm^{-2}$) is still better than the flux of unmodified membrane before and after (irreversible) protein adsorption (*i.e.*, 13.97 and 11.74 $m^3 \cdot m^{-2} \cdot hr^{-1}$ for unmodified membrane, and 15.44 and 14.29 $m^3 \cdot m^{-2} \cdot hr^{-1}$for modified membrane before and after irreversible protein adsorption, respectively). So, the total flux after (modification and irreversible protein adsorption) of the modified membranes is still better than the flux of the unmodified membrane.

SEM images of the unmodified PES membrane and modified PES membranes at different four modification conditions shown in Fig.5. The formed new layer on the top of the membrane surfaces is noticeable. The thickness of this new layer seems thicker at reaction temperature 40ºC than the thickness of layer formed at temperature25ºC. Also, using higher substrate concentration (15 mM 2-AP) gives the chance for increasing in the thickness of the formed layer especially at longer modification time (120 min). But in all cases, there is no homopolymer lumps are shown. This is because we use modifier with two legs that tends to grow in one dimension with formation a homogenous layer.

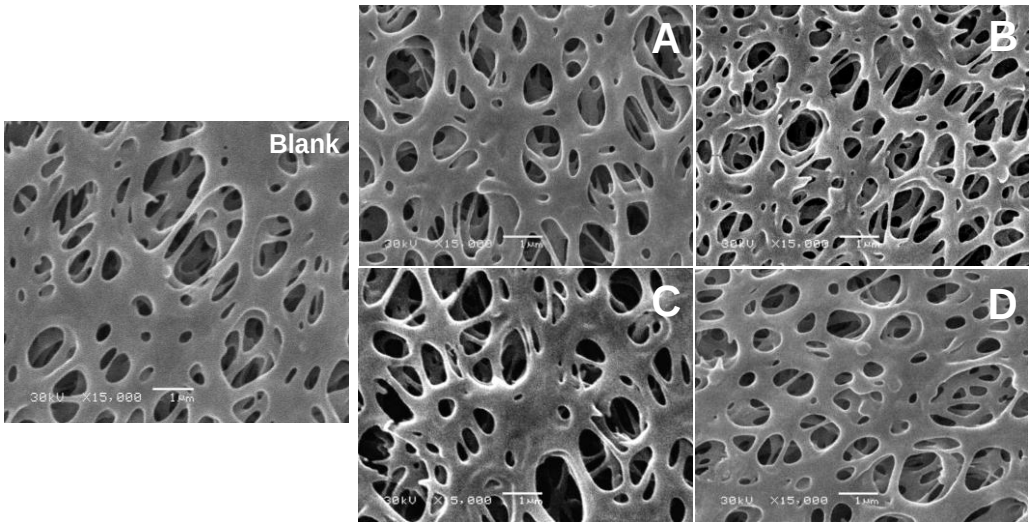

Fig. 5. SEM images (15000× magnification, scale bar is 1 µm) for blank membrane, and 2-aminophenol modified membranes: images A and B show modified membrane at 25ºC using 5 mM and 15 mM2-AP, respectively. Images C and D show modified membrane at 40ºC using 5 mM and 15 mM 2-AP, respectively. The reference reaction condition is 0.5 $U \cdot ml^{-1}$ enzyme, 120 min modification time, pH 5.5, and 0.1 M sodium acetate buffer.

From Fig. 6, it seems the modification does not affect harmfully on the mechanical properties of the modified membranes using 2-AP at different modification conditions used

in this study. Actually, we can see slightly improvement in the membrane strength at some modification conditions.

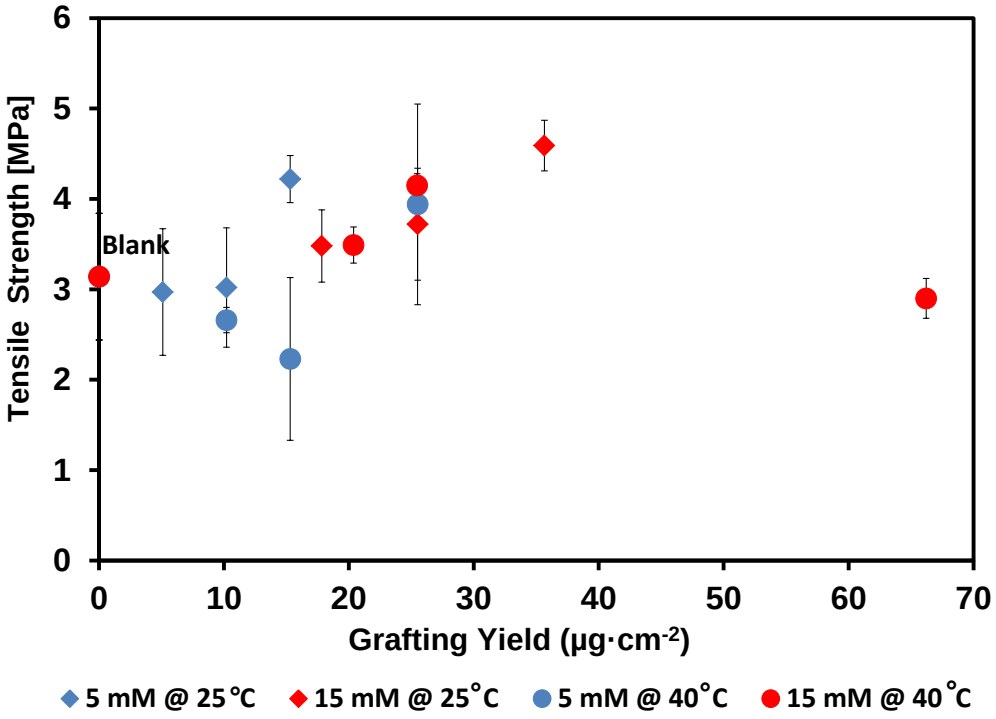

Fig. 6. Tensile strength of unmodified and modified membranes as a function of grafting yield; the reference reaction condition is 0.5 U·ml^{-1}laccase, pH 5.5, and 0.1 M sodium acetate buffer. 2-aminophenol (5 mM; blue and 15 mM; red) modifier is used at two reaction temperature (25ºC; diamond and 40ºC; circular) with different modification times (30, 60, and 120 min).

4. CONCLUSIONS

In this work, the 2-AP used to modify PES membranes using laccase bio-catalyst. This modification resulted in remarkable reduction in protein adsorption whereas the pure water flux increased. This modification doesn't affect harmfully on mechanical properties of the membrane. At different modification conditions, the formed modification layer seems homogenous all over the surface without formation of big lumps of homopolymers on the membrane surfaces as shown in our previous study using phenolic acids [Nady 2012]. The results of this work in full agreement with our investigation that the structure of the used modifier affect on the shape of the formed layer. The effect of this modification on the biofouling formation is currently carried out in our lab.

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