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Immobilization of Crude Polyphenol Oxidase Extracts from Apples on Polypyrrole as a Membrane for Phenol Removal

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Abstract

This research aims to make a polypyrrole (PPy) membrane and crude extract of polyphenol oxidase (PPO) as a membrane of mPPy/PPO apple extracts. The membrane of PPy/PPO-apple extract has been synthesized by the electrodeposition method. The electrolyte composition consists of a mixture of 0.10-0.20 M pyrrole (Py) and 50-100% PPO apple extract, which is stable using 50 mM of phosphate buffer solution at pH 6.80-7.00 and room temperature. The electrodeposition process is used 400 mesh steel gauze anode ST-304 and carbon plate cathode. Electrodeposition is carried out at potential = 5.00-6.00 V; current = 0.02-0.25 A; the distance from both electrodes = 1.00–2.00 cm for 300–500 seconds. The results from the deposition of PPy/PPO apple extract of the anode are a membrane of mPPy/PPO-apple extract, with total enzyme activity (U) = (957,1441, 2287 and 1754) using 2.00-5.00 mM phenol as a substrate which is measured based on the UV-visible spectrophotometric method. PPy and mPPy/PPO-apple extracts were characterized by SEM and SEM-EDS. The membrane of mPPy/PPO-apple extract can be used to remove phenol in industrial wastewater samples is 50-65% with a filtration capacity of 500 mL for 2 hours.

1. Introduction

Phenolic compounds and their derivatives are widely used as additives in several chemical and pharmaceutical industries. However, phenols are corrosive and toxic, which can pollute the environment. The increasing presence of phenols represents a significant environmental toxicity hazard; therefore, developing methods for removing phenols from industrial wastewater has generated significant interest [1]. The presence of phenols significantly reduces the biological degradation of other components [2]. According to Abd Gami [3], phenol's structure shows its reactivity, which leads to its properties like persistence in the environment, toxicity, and possible carcinogens against living organisms. The conventional methods of treating phenol waste are primarily chemical or physical, but this process creates a new secondary waste problem. For example, phenolic compounds obtained from industrial wastewater can be eliminated using activated carbon. Meanwhile, activated carbon is removed by combustion. During the combustion process under normal operating conditions, several of new compounds can be produced. These compounds are usually dioxins and furans which have an impact on human health [4]. Polyphenol oxidase (PPO) is a group of enzymes that catalyze the oxidation of phenolic compounds to produce a brown color on cut the surface of fruit and vegetables [5]. PPO catalyzes the oxidation of polyphenols to quinones which react non-enzymatically to produce colored pigments [6].

In plant cells, phenolic compounds are located in vacuoles, whereas PPO is located in plastids. Damaged areas in cells allow contact between PPO and phenolic compounds [7]. PPOs usually cause browning after cell damage due to aging, wounding, and the attack of pests and pathogens [8]. Apples that are mechanically damaged (peeled, bitten, and bumped) cause a browning reaction.

As apples matured, there was an increase in damaged cells around the larger intercellular spaces [9]. The apple fraction's chemical composition is related to its enzymatic browning, which assesses apple pomace's suitability for extracting phenolic compounds [10]. PPO activity is crucial to control enzymatic browning. The factors that influence PPO activity include the type and amount of endogenous phenolic compounds, the presence of oxygen, and the targeted pH to prevent enzymatic browning [11].

In this research, PPOs extract can be isolated from apples in phosphate buffer 50 mM pH 6.80-7.00 and room temperature. PPO performance will be more effective through immobilized on the surface of the polypyrrole (PPy) and synthesized by electrodeposition at optimum conditions [12]. The electropolymerization of pyrrole (Py) has been synthesized for membrane fabrication using the electrolysis technique. Some previous researchers have synthesized PPy by chemical oxidative polymerization technique at room temperature using monomer 0.10 M Py [13], 0.025, 0.05, 0.1, 0.15 M Py [14]. Immobilized enzymes have many operational advantages over free enzymes, such as reusability, enhanced stability, continuous operational mode, rapid reaction termination, easy separation of biocatalyst from the product, and reduced operating costs [15]. PPO has immobilized on PPy film by electropolymerization using cyclic voltammetry method on a platinum electrode (0.1 M Py and 2 µg/mL PPO in a 50 mM phosphate buffer solution pH 6.5 at 25℃) [12]. PPO immobilization was carried out during the electrosynthesis of PPy films by adding 100 µL of the crude extract of avocado in the electropolymerization medium containing 0.1 M $LiClO_4$ and 0.07 M Py [16]. PPO was immobilized in PEO/PPy and CP/Ppy [17]. PPy is very promising for commercial applications because of its good environmental stability, smooth synthesis and higher conductivity than many other conducting polymers [13]. The optimum conditions for the formation of the PPy/PPO films (the current density = 0.5 $mA.cm^{-2}$; a polymerization period = 150 s; 0.1 M Py and PPO activity = 50 $U.mL^{-1}$ [18]. The electropolymerization consisting 0.5 M Py is added to solutions of 1 M citric acid (pH 2.5), 0.25 M sodium acetate (pH 8.5), or 0.25 M sodium benzoate (pH 8.5) [19].

This research aims to make the membrane of PPy/PPO-apple extract (mPPy/PPO-apple extract), with high activity for industrial waste's degradation process, contaminated with phenol compounds and their derivatives. The PPO activity of the mPPy/PPO apple extract strongly correlates with the amount of PPO in apples. It depends on the synthesizing membrane process of PPy/PPO apple extract during electrodeposition.

2. Methodology

The chemicals, laboratory apparatus and instrumentations, and experiment procedure are explained in the sub-section below.

2.1. Equipment/Tool/Material

Chemicals. Green apples were obtained from the traditional market at Cimahi West Java Indonesia and frozen at 20°C. The reagents needed for the PPO apple extract were as previous researchers [20]. Other chemicals used were pyrrole (Py) 98% from Sigma Aldrich, potassium chloride (KCl), sodium dihydrogen phosphate (NaH_2PO_4), disodium phosphate (Na_2HPO_4), sodium hydroxide (NaOH), hydrochloric acid (HCl), phenol (C_6H_5OH), L-ascorbic acid ($C_6H_8O_6$), EDTA ($C_{10}H_{16}N_2O_8$), potassium permanganate ($KMnO_4$), oxalic acid dihydrate ($C_2H_6O_6.2H_2O$), and Biuret reagent (protein detection level 150-1.000 µg/mL). All solutions were prepared with double-distilled water.

Laboratory Apparatus and instrumentations. The equipment used was laboratory glassware, Hanna pH-meter New HI-98107, Mini Homogenizers T-18K SS Pestle, magnetic stirrer, GT-F1-ultrasonic cleaner with ABS housing, UV-Vis-NIR spectrophotometer (Shimadzu, Energy Dispersive X-Ray Spectroscopy (EDS or EDX) in conjunction with scanning electron microscopy (SEM)-JEOL JSM 6510 LA. It was also used one unit of electrolysis cell consisting of 500 mL of container cell, steel gauze with type ST-304 (1.50x1.50 cm), a carbon plate, and a set of cables. The electrolysis unit is connecting with a DC-power supply ATTEN APS-3005-DM30V/5A.

2.2. The synthesis of mPPy/PPO-apple extract

(a). The electrolyte solution is prepared by mixing 0.10-0.20 M Py solution and 50-100% PPO apple extract, then adding a 50 mM citrate buffer solution pH 6.80–7.00 in a 500 mL electrolysis cell;

(b). The next step is to prepare an ST-304 stainless steel anode (1.50x1.50 cm) and a carbon plate cathode, then connect it to a DC power supply (potential = 3.00-6.00 V; distance between the two electrodes = 1.00-2.00 cm and electrolysis time = 300-500 seconds); and

(c). The membrane characteristics determined were PPO activity, protein content, and PPO specific activity immobilized in the PPy matrix.

The effect of the potential variation (3.00-6.00 V) obtained four membrane variations: mPPy/PPO-apple extracts-1 (M-1); mPPy/PPO-apple extracts-2 (M-1); mPPy/PPO-apple extracts-3 (M-3), and mPPy/PPO-apple extracts-4 (M-4).

2.3. The Applications of mPPy/PPO apple extract

The application of mPPy/PPO-apple extract used the industrial sample containing phenol. The filtration unit was feed phase, membrane, permeate container, a pump, and manometer). A filtration set was combined with the mPPy/PPO-apple extract's housing membrane with a dead-end flow system and pressure (0.10-2.00 Bar) as the standard technique for microfiltration membranes [21]. The resulting permeate solution was collected in a permeate container.

2.4. Membrane Filtration Method

A microfiltration set consists of the feed phase, membrane housing, permeate container, manometer, and a pump.

3. Results and Discussion

3.1. The membrane of PPy/PPO-apple extract (mPPy/PPO-apple extract)

This research describes an effective process of synthesizing the mPPy/PPO-apple extracts using the electrodeposition method. The electrodeposition uses the steel gauze (1.50x1.50 cm) as an anode and a carbon plate as the cathode. The electrolysis was controlled by electrolytic optimum (the distance between the two electrodes = 1.50 cm and electrolysis time= 500 seconds). The composition of mPPy/PPO-apple extract has achieved 0.15 M Py and 75% (v/v) PPO-apple extract.

Physically, mPPy/PPO-apple extract can be seen in Fig. 1 (mPPy/PPO-apple extracts-1 (M-1); (mPPy/PPO-apple extracts-2 (M-2); (mPPy/PPO-apple extracts-3 (M-3), and mPPy/PPO-apple extracts-4 (M-4). However, the observations for these membrane images are not visible, and this will be discussed further in the SEM images for membranes.

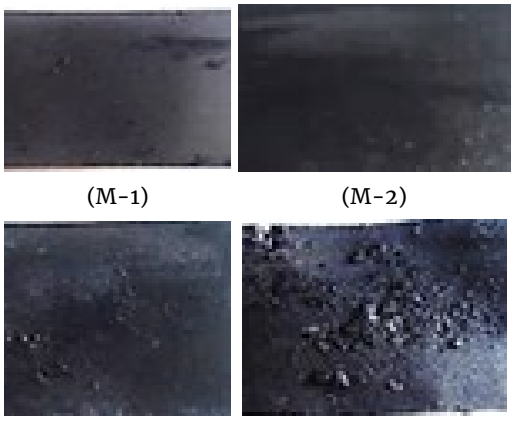


Figure 1. The profile of the mPPy/crude PPO apple extract membrane (mPPy/PPO-apple extracts-1 (M-1); (mPPy/PPO-apple extracts-2 (M-2); (mPPy/PPO-apple extracts-3 (M-3), and mPPy/PPO-apple extracts (M-4)

Table 1 shows the influence of potential (3,00-6.00 V) on membrane characteristics of mPPy/PPO-apple extract (M-1 to M-4), which were synthesized based on electrodeposition at optimum conditions (current, weight, and thickness membrane). The current response (0.04-0.22 A) shows the impact of the electrodeposition process. It is easy regulating during the PPO-apple extract is immobilization on PPy film growth at 3.00-6.00 V, so that it is as the difference in the shape the physically of the M-1 to M-4 of mPPy/PPO-apple extract (Figure-1). The potential increase in proportion to the increase in current. As explained by Wang [22], there is a relationship between current flow and mobility of PPy^+ and A^- ions (counterion), in which dopant anion concentration correlates with electrolyte solution consisting of Py and PPO in phosphate buffer pH 6.8-7.0. The amount of charge transported is also proportional to the electron transfer of the redox reaction between Py^+ and A^- from the 0.15 M PPy deposition process and the 75% (v/v) PPO extract-apple attached on the steel gauze anode. The other membrane parameters that can be studied in Table 1 are the observation digital multimeter measurement results. The thickness of mPPy/PPO-apple extract (mm) was 0.122-0.180; apple-extract PPy/PPO layer thickness (mm) was 0.038-0.092; so the predicted thickness of steel gauze (mm) was 0.084-0.088. Meanwhile, membrane weight (g) = 0.169-0.025. Assuming the M-3 membrane has the highest PPO activity (U) = 2287, when compared to M-1, M-2, and M-4, then M-3 is considered an ideal membrane (mass (g) = 0.173; thickness (mm) = 0.085; potential (V) = 5 and current (A) = 0.14) [23, 24].

Table 1. Measurement results for the synthesis of PPy/PPO apple-extract, with a composition of 0.15 M Py and 75% PPO apple extract synthesized by the electrodeposition method (steel gauze anode type ST-304, surface area 1.50 x 1.50 cm, and the carbon plate cathode; the distance between the two electrodes = 1.5x1.5 cm, and the electrolysis time is 500 seconds)

mPPy/PPO apple-extract	V (V)	I (A)	Weight (g)			Thickness (mm)			Total of PPO activity (U)
			steel gauze	mPPy/PPO apple-extract	PPy/PPO	steel gauze	PPy/PPO	mPPy/PPO apple-extract	
M-1	3.00	0.04	0.163	0.169	0.006	0.084	0.122	0.038	957
M-2	4.00	0.05	0.161	0.168	0.007	0.087	0.126	0.039	1441
M-3	5.00	0.14	0.163	0.173	0.011	0.085	0.132	0.047	2287
M-4	6.00	0.22	0.157	0.182	0.025	0.088	0.180	0.092	1754

The influence of the four different M1, M-2, M-3, and M-4 membranes formation, include the character of the steel gauze composition as supporting material during the electrodeposition process at a potential of 3.00-6.00 V. The results of previous research [25] that the steel gauze (ST-304) with the main component Fe (49.63%, b/b), and other components (Cr, Zn, Ni, Mo, Mn, Si, and Cu). The conditions for electrolysis also have an impact on the weight and thickness of the membrane. The PPy with Py oxidation occurring at the steel gauze anode and A as dopant anions [22], and the possible redox reactions that occur at steel gauze anodes and carbon plate cathodes are:

anode: $2Py(aq) + 2A^-(aq) \rightarrow 2Py^+A^-(s) + 2e^-$ (i)

cathode: $2H_2O(l) + 2e^- \rightarrow H_2(g) + 2OH^-(aq)$ (ii)

overall: $2Py(aq) + 2A^-(aq) + 2H_2O(l) \rightarrow 2Py^+A^-(s) + H_2(g) + 2OH^-(aq)$ (iii)

The quality of the film produced on electropolymerization depends on the interaction between polymer and dopant, which will direct the growth of polymer chains on the electrode's surface [26]. PPy can be used as a supporting material for PPO immobilization in apple extracts synthesized by electrodeposition technique. The electrochemical method involves the entrapment of biomolecules in organic polymers during the electro-generation of their electrode surfaces. The formation of polymers using electrolysis at a controlled potential of aqueous solutions containing monomers and biomolecules [27].

The stability of PPO (from apple extract) in a 50 mM phosphate buffer solution can affect the degradation process, so it is necessary to prepare it at optimum pH and room temperature and achieve the mPPy/PPO electrodeposition process. In Table 1, the achievement of PPO activity on the mPPy/PPO-apple extract membrane was 957-2287 U PPO activity before the electrodeposition process. In this process, PPO-apple extract has the

highest activity. Before the electrodeposition process, apple extract characteristics have been studied based on the PPO activity, the protein content, and the specific activity at the pH variation (6.00; 6.20; 6.40; 6.60; 6.80; and 7.00) are shown in Fig 2 and 3. In Fig 2, the highest PPO activity and total PPO activity is shown (U/mL)= 76.23 or (U)= 7623 in 100 mg apple extract; 2.5 mM phenol; pH 7 in a 50 mM phosphate buffer solution and room temperature).

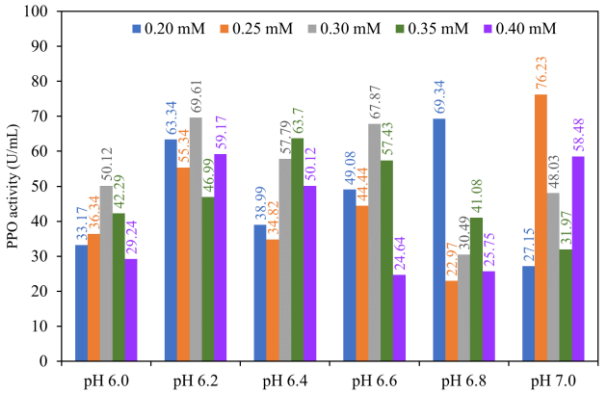


Figure 2. PPO activity in apple extract measured at various pH (6.00-7.00) using phenol (0.20-0.40 mM) as a substrate

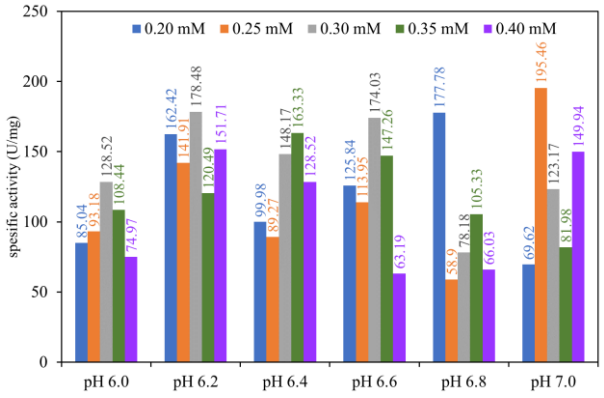


Figure 3. The specific activity of PPO in apple extract measured at various pH (6.00-7.00) using phenol (0.20-0.40 mM) as a substrate

A previous research [28] studied mPPO on Fuji apples purified by a combined protocol involving temperature induced phase partitioning (1.5% Triton X-100; 150 mL; total activity = 1,071,000 U; total protein = 157.50 mg, and specific activity = 6800 U/mg). Meanwhile, the research results of Queiroz *et al.* [6] have studied that PPO in cashew apple shows maximum activity (pH 6.5) and remains in the range of 63% (pH 7.0). PPO activity is not visible (pH above 7.0) because of the substrate oxidation. Cashew apple PPO showed the highest activity with o-dihydroxy-phenol catechol (100% of relative activity), followed by caffeic acid (18.5%), catechin (17.5%), and gallic acid (4.2%)

PPO activity was measured based on the spectrophotometric method and the initial rate of decrease in absorbance (phenol concentration) and the reaction time of quinone formation (curve at phenol λ_{max} = 282 nm; y = 1.2929 + 0.0041; R = 0.9970). Meanwhile, the protein content was (mg/mL)= 0.39; total protein (mg)= 39 or 0.4% in 100 g crude apple extract, which was measured based on the Biuret method (standard curve BSA λ_{max} = 278 nm; y = 0.0011x + 0.04; R = 0.9966). In Fig. 3, showing of the specific activity (U/mg)= 195.46 and total specific activity (U) = 19.5. PPO activity as a biocatalyst in a mPPy/PPO- apple extract can affect the function of phenol removal in industrial wastewater contaminated with phenol. Therefore, it needs to have a useful technique in making mPPy/PPO-apple extract during the electrodeposition process. It is necessary to ideal design between the filtration device unit and mPPy/PPO-apple extract for good performs smoothly in phenol's degradation process to a quinone. The PPO in a solution is deactivated by the presence of the reaction product. But, if it is immobilized, the separation of the product from this enzyme will reduce this inhibition. Immobilization can also increase stability and allow for continuous use of the enzyme [29].

SEM image at 250x magnification of morphology analysis of PPy and mPPy/PPO apple-extract for the M-3 (PPO activity (U) = 2287) has studied in Fig 4a and 4b. The morphology of the PPy and mPPy/PPO-apple extract films to clarify the observed PPy/PPO macromolecular structure from Fig 1 is unclear. In the SEM image of mPPy/PPO, there is a correlation between membrane pore size (about 10-20 µm) and a pressure of 0.5 Bar, and this is still close to the standard technique for the membrane microfiltration group (dead-end system) according to Mulder [21]. The thin film of PPy has uniform granular morphology and average grain size of ~ 0.7 µm [13]. The research results from Yussuf *et al.* [14] have studied the SEM photograph of PPy films has been made of Py (0.05 M)/$FeCl_3$ (0.1 M), which shows the fibrillar morphology. While the SEM image of PPy films that Py has made (0.05 M)/$N_2H_8S_2O_8$ (0.1 M) showed spherical morphology. Accordingly, changes in the morphology of PPy are very dependent on the oxidant type used during the chemical polymerization. PPy morphology structure significantly effects its conductivity. It was found that the fibrillar morphology sample have higher conductivity than the sample with a spherical morphology. Based on these assumptions, if we observe the SEM images in Fig. 4a and 4b on the micrograph of PPy and PPy/PPO, both of them are more likely to show spherical morphology with low conductivity. However, it is necessary to evaluate the results of mPPy/PPO-apple extract performance against sample solution contaminated with phenol in the subsequent discussion.

Meanwhile, in Fig. 5a and 5b, we have studied the SEM-EDS profile of PPy, and mPPy/PPO-apple extract for the M-3 (PPO activity (U) = 2287) with 1000x magnification. SEM-EDS images of PPy and mPPy/PPO-apple extracts were observed with the main component C in PPy and the main component Fe in steel gauze. C's element has increased the amount of C (0.277 keV) from 27.44 to 28.67%. This is possible because the PPy will be more dominant with the polymer's growth covering the steel gauze's surface as mPPy/PPO-apple extract. For this reason, the SEM-EDS profile of PPy to mPPy/PPO-apple extract also has an impact on steel gauze as the main support for the membrane. Fe is the main component in

steel alloys, so that the mass scale decreases, Fe (6,398 keV) from 35.06 to 34.48% (b/b). The other components in the stell gauze alloy also decreased of the mass scale such as Cr (5.411 keV) from 10.39 to 10.29% (b/b); Ni (7.47 keV) from 6.47 to 6.28% (b/b);; Si (1.739 keV) from 0.37 to 0.29% (b/b);. Mo (2.293 keV) profile = 1.32% (b/b); in PPy SEM-EDS and Mo component is the part of the content in the steel gauze alloy. The Mo component in SEM-EDS of mPPy/PPO-apple extract does not appear after PPy was immobilized PPO-apple extract. In this condition, the possibility of Mo as a small component contained in the steel gauze has been covered by the appearance like Al component (1.486 keV) = 0.22% (b/b); and K = (3.312 keV) = 0.12% (b/b). Whereas the presence of the Cl (2,621 keV) component, amounting to 1.27-2.24% (w/w), can be obtained as a byproduct of the electrolysis process. The chlorine gas (Cl_2) is derived from KCl, the supporting electrolyte during the PPy electropolymerization process. The atomic component of oxygen with a composition of 17.0% (b/b), which binds to Cu metal (8.040 keV) = 0.41% (b/b); and Cu as the active site of PPO, can also be detected by SEM-EDS of mPPy/PPO-apple extract. The presence of the Cu component has confirmed the existence of PPO as a biocatalyst in the M-3 membranes.

3.2. The Applications of mPPy/PPO apple extract

The mPPy/PPO-apple extract (M-1–M-4) has been applied to an industrial sample's initial solution containing 1.00 mM of phenol. The results of a permeate solution containing phenol remeasured phenol concentration in the final solution (Fig. 5a and 5b). Based on Fig 5, it is obtained the absorbance (A) measurement results at phenol λ_{max} = 278 nm, (A) vs. mPPy/PPO-apple extract (M-1–M-4). The initial phenol (1.00 mM) in the feed phase has decreased after the sample solution's filtration process containing phenol from the permeate phase, which was flowed using a mPPy/PPO-apple extract (Fig. 6). The decreases of absorbance from phenol-1 (A=1.470; 1.0 M phenol) as the initial phenol in the feed phase and it was applied to four membranes (in the axis of mPPy/PPO apple-extract (M-1-M-4). Thus, phenol oxidation to quinone increases after the flow of phenol is through the membrane, and a little phenol is produced in the permeate solution. This is possible in the permeation process and membrane function on the role of PPO as an immobilized biocatalyst in PPy. The performance of the membrane in the phenol degradation process of the four membranes (M1=48,6; M2=51,2%; M3= 64,9% and M4= 58,2%) for a filtration capacity of 500 mL for 2 hours. The ideal membrane performance is required to observe the electrodeposition process in membrane synthesis with electrolytic conditions (potential, two electrode distance, and time) and electrolyte composition (PPy and PPO-apple extracts). A previous researcher [28] has studied two industrial wastes treated after dilution (1/10); with PPO (140 U) immobilized on IPS 748 membranes.

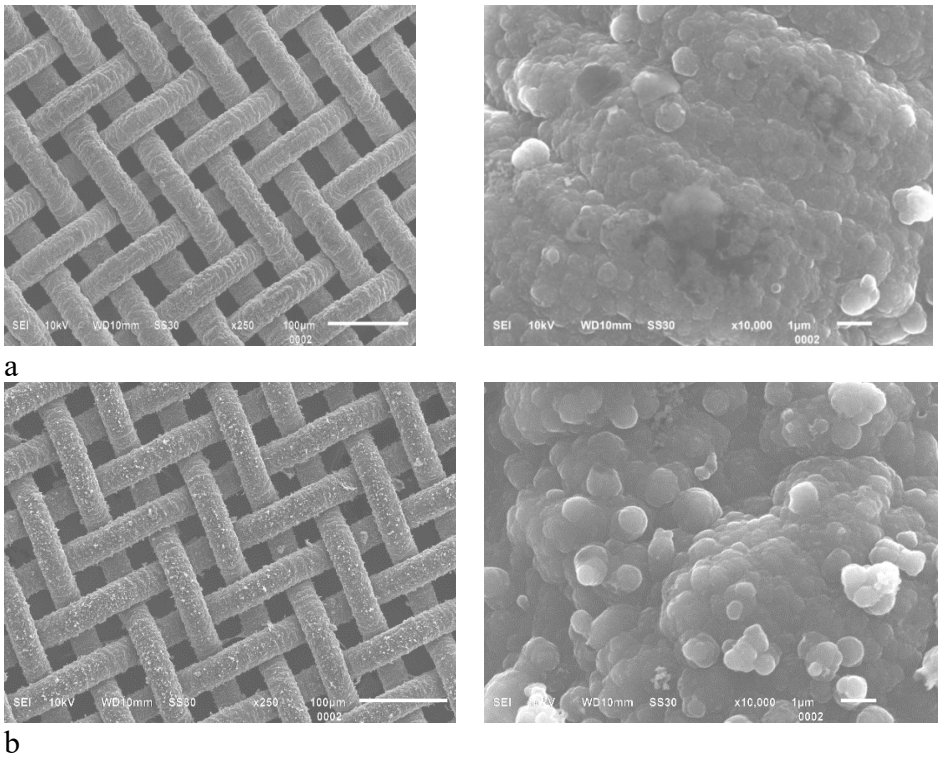
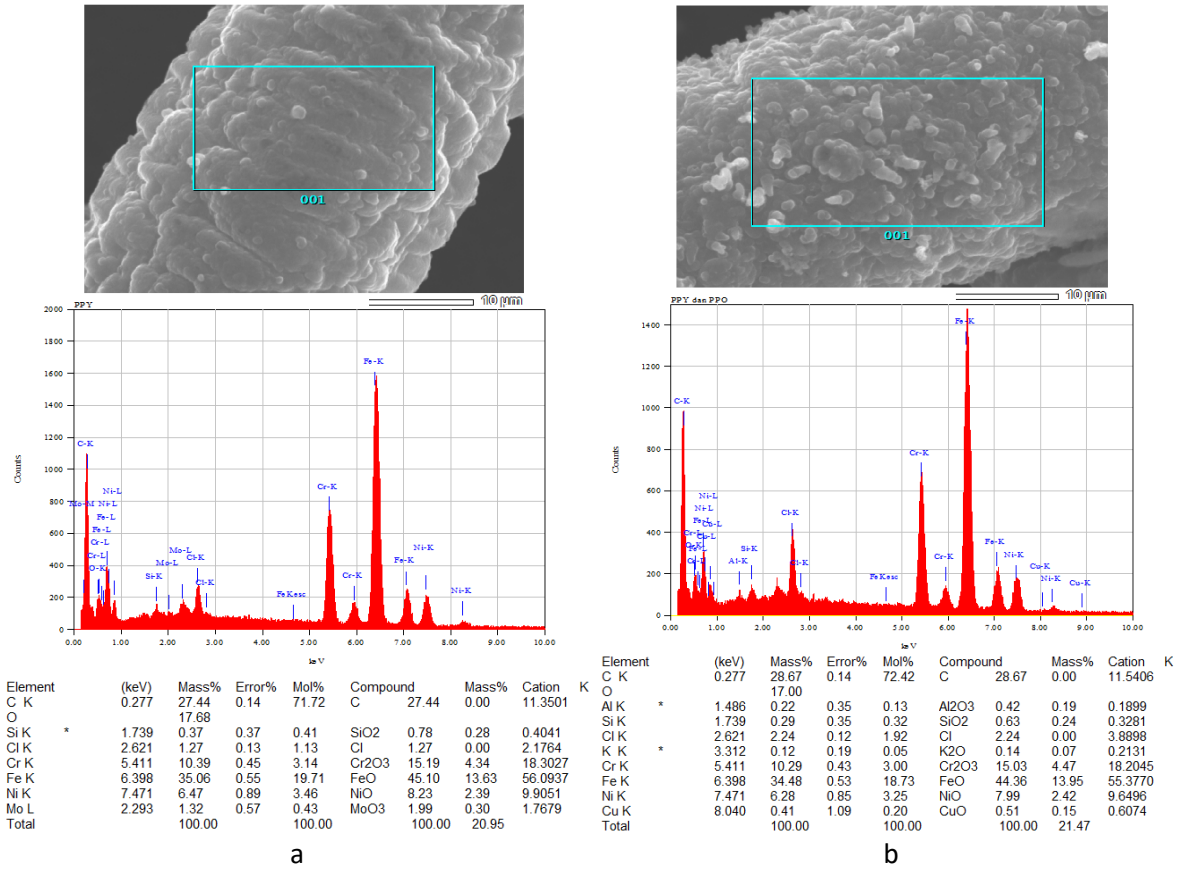


Figure 4. SEM image with 250x (left) and 1000x (right) magnification for morphology analysis: (a) PPy thin film and (b) mPPy/PPO apple-extract for the M-3 membrane with PPO activity (U) = 2287

For phenol wastes (the initial phenol concentration = 2,00 mM), 100% phenol conversion was successfully (for 2 hours at pH = 5.0). Phenol efficiency was 50 mL in 10 mg phenol/L as an artificial waste solution can be removed, i.e., 86% for 24 hours at 25°C using a membrane (1 g PPO) mobilized into a chitosan-SiO_2 gel) [30]. Immobilization of PPO on chitosan-coated capillary membranes by cross-flow circulation in 0.1 M K_2HPO_4/KH_2PO_4 buffer (pH 6.80) on the shell (outer) side of the membranes, for 1 hour at 25°C [29]. An immovable cell membrane bioreactor was prepared to investigate high concentration phenol removal using Pseudomonas putida American Type Culture Collection 49451. Phenol removal was obtained in 95 hours, whereas there was no cell

growth, and phenol degradation was observed in a free suspension system at 1.00 mg/L phenol, from an initial phenol of 1.20 mg/L [4]. The quantification of total polyphenols or single subclasses is carried out primarily by photometric measurements, either with or without prior derivatization [31].



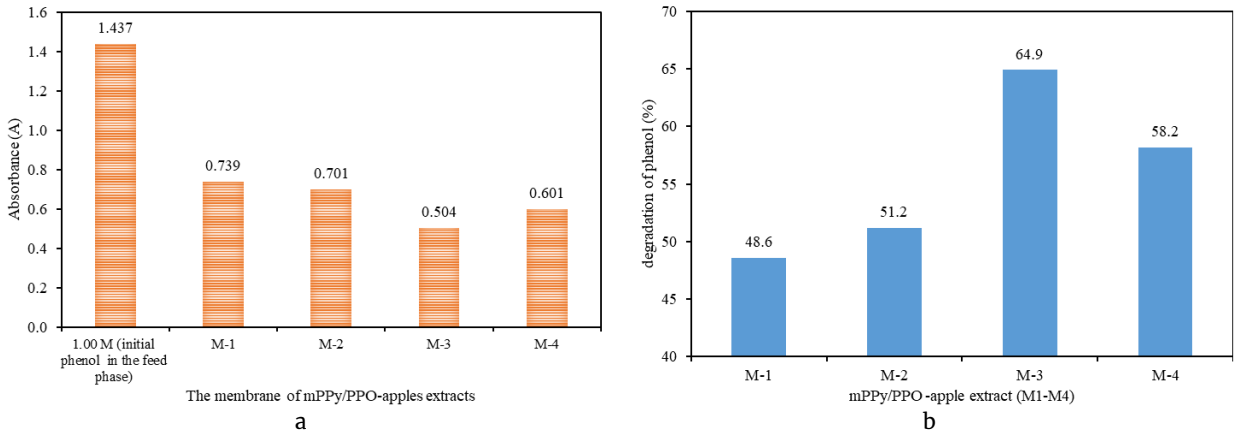
Element		(keV)	Mass%	Error%	Mol%	Compound		Mass%	Cation	K
C K		0.277	27.44	0.14	71.72	C	27.44	0.00	11.3501	
O			17.68							
Si K	*	1.739	0.37	0.37	0.41	SiO2	0.78	0.28	0.4041	
Cl K		2.621	1.27	0.13	1.13	Cl	1.27	0.00	2.1764	
Cr K		5.411	10.39	0.45	3.14	Cr2O3	15.19	4.34	18.3027	
Fe K		6.398	35.06	0.55	19.71	FeO	45.10	13.63	56.0937	
Ni K		7.471	6.47	0.89	3.46	NiO	8.23	2.39	9.9051	
Mo L		2.293	1.32	0.57	0.43	MoO3	1.99	0.30	1.7679	
Total			100.00		100.00		100.00	20.95		

a

Element		(keV)	Mass%	Error%	Mol%	Compound		Mass%	Cation	K
C K		0.277	28.67	0.14	72.42	C	28.67	0.00	11.5406	
O			17.00							
Al K	*	1.486	0.22	0.35	0.13	Al2O3	0.42	0.19	0.1899	
Si K		1.739	0.29	0.35	0.32	SiO2	0.63	0.24	0.3281	
Cl K		2.621	2.24	0.12	1.92	Cl	2.24	0.00	3.8898	
K K	*	3.312	0.12	0.19	0.05	K2O	0.14	0.07	0.2131	
Cr K		5.411	10.29	0.43	3.00	Cr2O3	15.03	4.47	18.2045	
Fe K		6.398	34.48	0.53	18.73	FeO	44.36	13.95	55.3770	
Ni K		7.471	6.28	0.85	3.25	NiO	7.99	2.42	9.6496	
Cu K		8.040	0.41	1.09	0.20	CuO	0.51	0.15	0.6074	
Total			100.00		100.00		100.00	21.47		

b

Figure 5. SEM-EDS image at 1000x magnification for chemical component analysis: (a) PPy thin film and (b) mPPy/PPO apple-extract for the M-3 with PPO activity (U) = 2287



a b

Figure 6. The results of the permeate solution containing phenol were remeasured the phenol concentration in the final solution: The profile of A (phenol λ_{max} = 278 nm) vs. mPPy/PPO apple-extract (M-1–M-4). The decreases of absorbance, from phenol-1 (A=1.470) as the initial phenol in the feed phase = 1.00 mM), and it was applied to four membranes (in the axis of mPPy/PPO apple-extract (M-1–M-4); The performance of the membrane in the phenol degradation process of the four membranes (M-1=48,6; M-2=51,2%; M-3= 64,9% and M-4= 58,2%) for a filtration capacity of 500 mL for 2 hours.

4. Conclusion

The membrane of mPPy/PPO-apple extract (0.10-0.20 M (Py) and 50-100% PPO apple extract), which has a high PPO activity, supports industrial waste degradation contaminated with phenol compounds and their derivatives. The membrane of PPy/PPO apple extract has been synthesized by the electrodeposition method (potential = 3.00-6.00 V; current = 0.02-0.25 A; the distance from both electrodes = 1.00-2.00 cm for 300-500 seconds; PPO activity (U/mL) = 30.00-60.00 ; total PPO (U) = 3000-6000 U; phenol 2.00-5.00 mM as a substrate). The membrane of mPPy/PPO apple extract can be used to degrade industrial wastewater contaminated with phenol. The membrane's performance in the phenol degradation process of the four membranes is 30-40% for a filtration capacity of 500 mL for 2 hours.

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