IMMOBILIZATION OF CRUDE POLYPHENOL OXIDASE PURPLE EGGPLANT EXTRACT ON CHITOSANMEMBRANE FOR REMOVAL OF PHENOL WASTEWATER

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Abstract: This research studied the synthesis of chitosan (CS) membrane with the basic component of polyphenol oxidase (PPO) from purple eggplant extract added with Cu2+ ions. The activity of the immobilized PPO in the CS- membrane was determined by the UV-visible spectrophotometer methods. The results showed that the membrane characteristics of CS-/PPO-Cu2+ purple eggplant extract: (diameter = 9 cm, thickness = 0.021 cm; PPO activity= 2,894.99 U). The performance of the membrane is designed as filtration with a dead-end system, with three times the filtration process, and ten times the use of a batch system membrane. The application of the CS-crude PPO- Cu2+ membrane was carried out on samples of artificial waste and textile waste solutions. The results achieved from the samples of artificial waste and textile waste, respectively, (the rejection = 68.77% and 63.69%; the conversion of phenol into catechol = 16.92% and 17.19% ; the conversion of phenol to quinone = 42.96% and 42.64%; flow rate = ±11 L/h and ± 5 L/h; membrane flux= 10,007.99 L/m2.h at a pressure of 1 bar; permeability= 300–1,000 L/m2.h.bar). The membrane lifetime is stable up to 8 times use from 10 times use.

Keywords: chitosan, membrane, polyphenol oxidase, purple eggplant extract

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INTRODUCTION

The presence of phenolic compounds is a worldwide problem due to natural and chemical processes originating from industry or human activities. Exceeding the standard level in our environment is not a new problem today, especially in developed or developing countries [1]. Based on the Regulation of the Minister of the Environment of the Republic of Indonesia Number 5 in 2014 for Wastewater Quality Standards, it is stated that the limit of phenol content in liquid waste for textile industry waste treatment activities is 0.5 ppm [2]. One of the most effective phenol removal techniques from wastewater is enzymatic treatment. Based on the results of previous studies Polyphenol Oxidase (PPO) can be applied to phenol-contaminated wastewater [3]–[6]. PPO oxidizes phenol to o-quinone via catechol, then undergoes non-enzymatic polymerization to form water-insoluble aggregates [3]. The use of membrane technology to remove particulates, microorganisms, and colloidal matter from industrial waste streams have been widely developed. The microfiltration pore size (M) usually varies from 0.1 to 10 nm. The separation mechanism is not simple because particles smaller than the pore size will flow through the pores while larger particles will be retained on the membrane surface [7]. Chitosan (CS) is a relatively inexpensive and abundant polymer and has been widely studied in its recent applications in membrane science. Chitosan is a derivative of chitin as the second most abundant biopolymer and shows many advantages needed to be synthesized into a membrane [8], as has been successful based on previous papers . One article has discussed showing the great potential of CS-based membranes modified by polyethylene oxide (PEO) and glutaraldehyde which can reduce pore size, increase tensile strength, and thermal stability of cross-linked CS/PEO [9]. Applications include gas separation, direct methanol fuel cell, pervaporation, water treatment and biomedical fields [8].

Immobilized PPO may be useful for the removal of phenolic compounds from industrial wastewater. PPO from potato was immobilized in crosslinked chitosan– SiO_2 gel and used to remove phenol [10]. The ability of enzyme immobilization on polypyrrole membranes has been studied for industrial waste applications containing phenol [11], [12] and we have done PPO immobilization on a polymer matrix in a previous study [5]. This research relates to the preparation of chitosan and polivinil alcohol (CS/PVA) membrane that immobilized PPO from purple eggplant crude extract, then added Cu^{2+} ions as CS/PPO- Cu^{2+} membrane. The CS/PPO- Cu^{2+} membrane was then used for degradation of phenol-contaminated industrial wastewater. The presence of Cu^{2+} in the purple eggplant PPO-Cu^{2+} may contribute to improving the performance of the CS/PPO-Cu^{2+} membrane in the removal of phenol needed as a filtration process [13]. We have done PPO immobilization on a polymer matrix in a previous study [11]

EXPERIMENTAL SECTION

Materials and Methods

Chemicals: The crude extract of polyphenol oxidase from purple eggplant was obtained following the procedure of our previous research [5]. Chitosan, acetic acid (CH_3COOH), polyvinyl alcohol (PVA), glutaraldehyde ($C_5H_8O_2$), acetone (CH_3COCH_3), sodium hydroxide (NaOH), ascorbic acid ($C_6H_8O_6$), sodium citrate ($C_6H_5O_7Na_3.2H_2O$), citric acid ($C_6H_8O_7.H_2O$), catechol ($C_6H_6O_2$), copper (II) sulfate ($CuSO_4$) and distilled water. The samples of industrial effluent were obtained from the sewage inlet of a wastewater treatment plant

Laboratory Apparatus and instrumentations: laboratory glassware. Instruments used: analytical balance Mettler AE-200, magnetic stirrer, Eppendorf micropipette, pH-meter Checker Hanna, ultrasonic cleaner Branson 3510, Fourier Transform Infra-Red (FTIR) Prestige 21 Shimadzu, Japan detector MTG-21 and Scanning Electron Microscope, SEM JEOL JSM- 6510 LA.

General Procedure

Preparation of CS(1-5%)-PVA membrane and CS(4%)-PVA-PPO-Cu^{2+} membrane Chitosan membrane is made by mixing two solutions with a ratio of 1: 1, namely chitosan in acetic acid (1 to 5%) and 1% polyvinyl alcohol solution. The mixture was homogenized with a magnetic stirrer while adding 2-5 mL of 4-6% glutaraldehyde. The solution was left for ± 24 hours, then printed on a petri dish (diameter = 9 cm). The membrane was immersed in a 1% NaOH solution, washed the chitosan membrane with aquades, then dried. Then, CS(4%)- PVA-PPO-Cu^{2+} was prepared by immersing (± 24 hours) CS-(4%)-membrane into 80 mL of purple eggplant crude extract PPO solution and adding Cu^{2+} ions.

Application of the CS(4%)-PVA-PPO-Cu^{2+} membrane to samples

A set of filtration equipment is prepared to carry out the filtration process for the sample solution (artificial wastewater and textile wastewater). The sample solution in a 500 mL feed container is passed through a filtration device with four membrane dead-end module systems. Other unit equipment uses a pump at a pressure of 1 bar during the filtration. In the final result of filtration, the solution is accommodated in a permeate container. This filtration system is carried out 10 times with a batch system.

Detection Method

Physical tests of CS(1-5%)-PVA membrane and CS(4%)-PVA/PPO-Cu^{2+} membrane included: mass, thickness, and degree of swelling.The activity of PPO on catechol substrates was carried out by spectrophotometric method based on the decrease in absorbance concentration to changes in catechol concentration [5].The calculations of the physical quality of the membrane include flux, permeability, and rejection [18]. The flux (J) depends on the membrane surface area (A) and the change in volume with time (dV/dt) as shown in the following equation:

$$J=1/A\ dV/dt \qquad (1)$$

Phenol rejection is the difference between the concentration of phenol in the feed phase (Cf) and the concentration of phenol in the permeate phase (Cp) and divided by the concentration of phenol in the feed phase (Cf):

$$Rejection =1- Cp/Cf \times 100\% \quad (2)$$

RESULTS AND DISCUSSION

Characteristics of CS-PVA And CS-PVA/PPO-Cu^{2+} Membranes

The characteristics of the CS(1-5%)-PVA membrane are strongly influenced during the membrane manufacturing process and the composition between chitosan (CS) and polyvinyl alcohol (PVA). The addition of PVA to the CS-membrane adds mechanical properties because PVA is a biodegradable, biocompatible, and non-toxic synthetic polymer with excellent film-forming properties [8]. Membranes with more chitosan composition have a small water uptake value because chitosan is insoluble in water. The presence of hydrogen bonds that are too strong between the amine group (-NH_2) and the hydroxyl group (-OH), so the addition of hydrophilic PVA into the membrane increases the percentage of water uptake. The addition of glutaraldehyde functions as a crosslinking agent, it is possible that some of the hydroxyl and amine groups are bonded to each other, and the other part between the hydroxyl and amine groups is still present for proton exchange. The solution obtained before printing must be left for approximately 24 h to remove trapped air bubbles during membrane printing which can cause holes in the membrane, but it can also cover the membrane pores. In another paper, it has been reported that PVA/CS porous composite hydrogels with a ratio of 6:4 exhibited can be good candidates for tissue engineering materials in cartilage repair [14].

The purpose of the membrane drying process in an oven (temperature 60-80oC; for 2-4 h) after the membrane is printed is to remove excess water content in the membrane. Treat the addition of 1% NaOH until the membrane is submerged (about 24 h), and finally, the membrane can be lifted to the surface. It is expected that the 1% NaOH solution can diffuse to the bottom of the membrane so that the membrane will be pushed up and peeled off. Furthermore, repeated washing of the membrane with distilled water is intended to remove the remaining NaOH [15], [16].

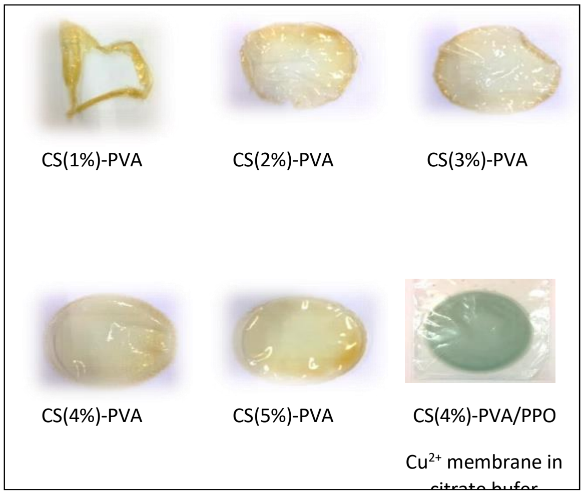


Figure 1. Profile of CS(1-5%)-PVA and CS(4%)- PVA/PPO-Cu2+ membranes

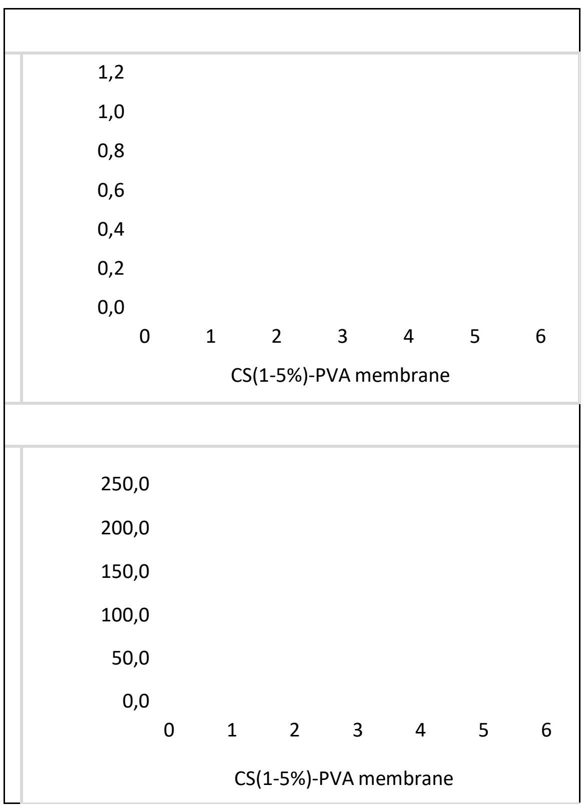


Figure 2. Density and degree of swelling of CS(1- 5%)-PVA membranes

The physical form of CS(1-5%)-PVA and CS(4%)- PVA/PPO-Cu2+ membranes is shown in Figure 1. In fact, for CS(1-3%)-PVA membrane, the condition is more easily torn and brittle, while for CS(5%)-PVA membrane it is thicker and the relative mechanical strength is higher than other membranes. This condition of CS(5%)-PVA requires a long time for the immobilization process in immersion in PPO-Cu2+ solution. Thus, CS(4%)-PVA membrane is considered a candidate for chitosan membrane which is quite ideal for the immobilization process in the enzyme solution. The density of the CS(1-5%)-PVA membrane was greater based on increasing the solvent concentration of acetic acid from 1 to 5% in both wet and dry conditions (Fig. 2). Characteristics of CS(4%)-PVA membrane with a higher density of 1.669 ± 0.108 in the wet state which contains a certain amount of water and the density decreases in the CS(4%)-PVA membrane in the dry state (0.872 ± 0.085) which is narrower, due to the concentration of reduced water. Meanwhile, the presence of PPO from purple eggplant extract and the addition of Cu2+ ions contributed to the density of the CS(4%)-PVA/PPO-Cu2+ membrane (0.881 ± 0.088).This is as described in our previous research which discusses [12].

Storage of this membrane is safer in 0.5 M citrate buffer with a pH of 7.0 so that the enzyme remains stable and does not undergo denaturation immobilized in CS(4%)-PVA (Fig. 1). PPO activity in PPO-Cu2+ immobilized purple eggplant in chitosan membrane was measured based on the action of PPO on catechol substrate using UV-Vis spectrophotometer. Based on the initial rate method as the correlation between absorbance and time obtained Y = 1.4775 x + 0.0474; R2 = 0.9989; crude PPO □max = 226.30 nm. The rate of the enzymatic reaction is expressed by the slope of thecurve with decreasing substrate concentration with time. Enzyme activity with the addition of Cu metal ions will add greater activity than without of Cu2+ activator as reported by previous studies [13]. The activity of immobilized PPO on CS(4%)-PVA was 36.94% or 2894.99 U in 8 mM catechol as a substrate.

Table 1. Characteristics of CS(4%)-PVA and CS(4%)-PVA/PPO- Cu2+ membranes

Membrane	Mass (g)	Thickness (cm)	Volume (cm3)	Density (g/cm3)	PPO activity (U)	Activity of immobilized PPO in membranes (%)	Swelling (%)
CS(4%)-PVA (wet membrane)	2.100 ±0.028	0.020 ±0.001	1.262 ±0.079	1.669±0.108			155.87±9.203
CS(4%)-PVA (dry membrane)	0.848 ±0.008	0.015 ±0.002	0.982 ±0.102	0.087±0.085			
CS(4%)-PPO-Cu2+ (dry membrane)	0.868±0.0104	0.016 ±0.002	0.996 ±0.109	0.881±0.088	2,894.99±1,742.67	36.94	0.881±0.088

According to Mulder [17], that the thickness of the symmetrical membrane (porous or non-porous) ranges from 10 to 200 µm, which affects the mass transfer resistance, and decreasing the thickness of the membrane results in an increase in the permease rate. In the results of the study, the low thickness of the chitosan membrane ranged from 16 µm. Testing the degree of swelling on the membrane aims to predict the size and ability of a particular solvent or substance to be able to diffuse into the membrane. Swelling can also indicate that there is still a cavity between the bonds in the polymer, which this cavity can affect the mechanical properties of the polymer, the smaller the cavity, the higher the mechanical properties. The results of the swelling test can also be observed on the basis of the concentration of chitosan, namely the higher the concentration of chitosan in the membrane, the lower the degree of swelling.

The higher the concentration of chitosan, the distance between the chitosan molecules will be closer and the pores formed on the membrane will be smaller so that it is difficult for water to diffuse into the membrane which causes the ability to expand is small. On the other hand, the lower the concentration of chitosan in the membrane, the greater the swelling ability. At a small concentration of chitosan, it is possible that standard curve Y = 0.2155x + 0.0347; R2 = 0.9996). more solvents are used or the less solute is present, the larger the pores of the membrane formed. Meanwhile, the CS(4%)-PVA/PPO-Cu2+ membrane with 5% chitosan had a lower degree of swelling, meaning that the pores were too small and the membrane filtration performance process would be slower. The membrane of CS(4%)-PVA/PPO-Cu2+ is considered optimum with a high concentration having the lowest degree of swelling, it is expected that in the filtration process, the membrane has better resistance in solution [18].

Application of CS(4%)-PVA/PPO-Cu2+ membrane to textile wastewater

The CS(4%)-PVA/PPO-Cu2+ membrane was further designed as a dead-end system filtration, with three times of filtration, and ten times of using batch system membranes (Table 1). The membrane of CS(4%)- PVA/PPO-Cu2+ obtained rejection = 63.69% and membrane lifetime is stable up to 8 uses out of 10 trials. The results obtained, were conversion of phenol to catechol = 17.19% and conversion of phenol to quinone = 42.64%. Experimental results obtained the conversion of phenol (phenol λmax = 269.8 nm on the standard curve Y = 0.2004x - 0.0033; R2 = 0.9996), into catechol (catechol λmax =

275.8 nm on the standard curve Y = 0, 2134x + 0.0229; R2 = 0.9998).
Meanwhile, the conversion of phenol to quinone (quinone λmax = 246.1 nm on the

Table 2. Calculation results of conversion of phenol to catechol, conversion of phenol to quinone and rejection of phenol with 500m mL of artificial initial waste containing phenol of 2.34 ppm and 500 mL of textile wastewater containing phenol of 1.99 ppm. The CS(4%)-PVA/PPO-Cu2+ membrane is designed as a dead-end system filtration, with three filtration treatments, and ten times the use of batch system membranes

use of CS(4%)-PVA/PPO-Cu2+ membranes in the filtration system	Artificial wastewater samples					
	phenol		catechol		quinone	
	(ppm)	Rejection (%)	(ppm)	conversion of phenol to catechol (%)	(ppm)	conversion of phenol to quinone (%)
1	0.763±0.021	67.38	0.380±0.008	16.20	0.980±0.008	41.947
2	0.757±0.021	67.66	0.377±0.021	16.14	0.973±0.039	41.510
3	0.793±0.012	66.10	0.343±0.017	14.76	0.933±0.012	39.967
4	0.853±0.025	63.53	0.317±0.017	13.55	0.897±0.019	38.277
5	0.930±0.022	60.26	0.290±0.008	12.47	0.850±0.008	36.247
6	0.987±0.012	57.83	0.270±0.008	11.57	0.810±0.022	34.557
7	1.030±0.022	55.98	0.250±0.014	10.66	0.743±0.021	31.710
8	1.100±0.022	52.99	0.253±0.005	10.84	0.707±0.026	30.167
9	1.190±0.024	49.15	0.213±0.012	9.10	0.620±0.049	26.590
10	1.333±0.017	43.02	0.163±0.012	7.11	0.537±0.033	22.920
use of CS(4%)-PVA/PPO-Cu2+ membranes in the filtration system	Textile wastewater samples					
	phenol		catechol		quinone	
	(ppm)	Rejection (%)	(ppm)	conversion of phenol to catechol (%)	(ppm)	conversion of phenol to quinone (%)
1	0.737±0.012	62.98	0.330±0.008	5.95	0.777±0.009	40.13
2	0.753±0.017	62.14	0.327±0.009	5.67	0.790±0.057	38.85
3	0.767±0.021	61.47	0.327±0.005	5.73	0.757±0.040	38.14
4	0.803±0.017	59.63	0.317±0.005	5.51	0.750±0.050	37.64
5	0.823±0.017	58.63	0.287±0.005	5.07	0.793±0.042	39.43
6	0.877±0.005	55.95	0.253±0.009	4.48	0.760±0.033	38.05
7	0.967±0.021	51.42	0.210±0.008	3.67	0.737±0.025	36.18
8	1.203±0.009	39.53	0.183±0.005	3.36	0.577±0.017	29.43
9	1.370±0.008	31.16	0.167±0.009	3.07	0.367±0.025	20.60
10	1.467±0.012	26.30	0.133±0.009	2.41	0.327±0.025	18.04

The operational parameters of the membrane include: flow rate = ± 5 L/h; flux = 10007.99 L/m2.h at a pressure of 1 Bar; and permeability= 300–1000 L/m2.h.Bar. Based on the data above, the removal of phenol in wastewater is considered more effective by filtration using a CS(4%)-PVA-PPO-Cu2+ membrane. Based on the permeability value of the membrane performance, the CS(4%)-PVA/PPO-Cu2+ purple eggplant membrane tends to be an ultrafiltration membrane, with a permeability of 300 to 1000 L/m2.h.Bar. The ability of a membrane to filter certain components from a solution is the most important parameter in determining the type of membrane selected. A good membrane can produce high and stable permeability as a function of time. This membrane has the potential to reduce COD and BOD values in textile industrial waste samples.

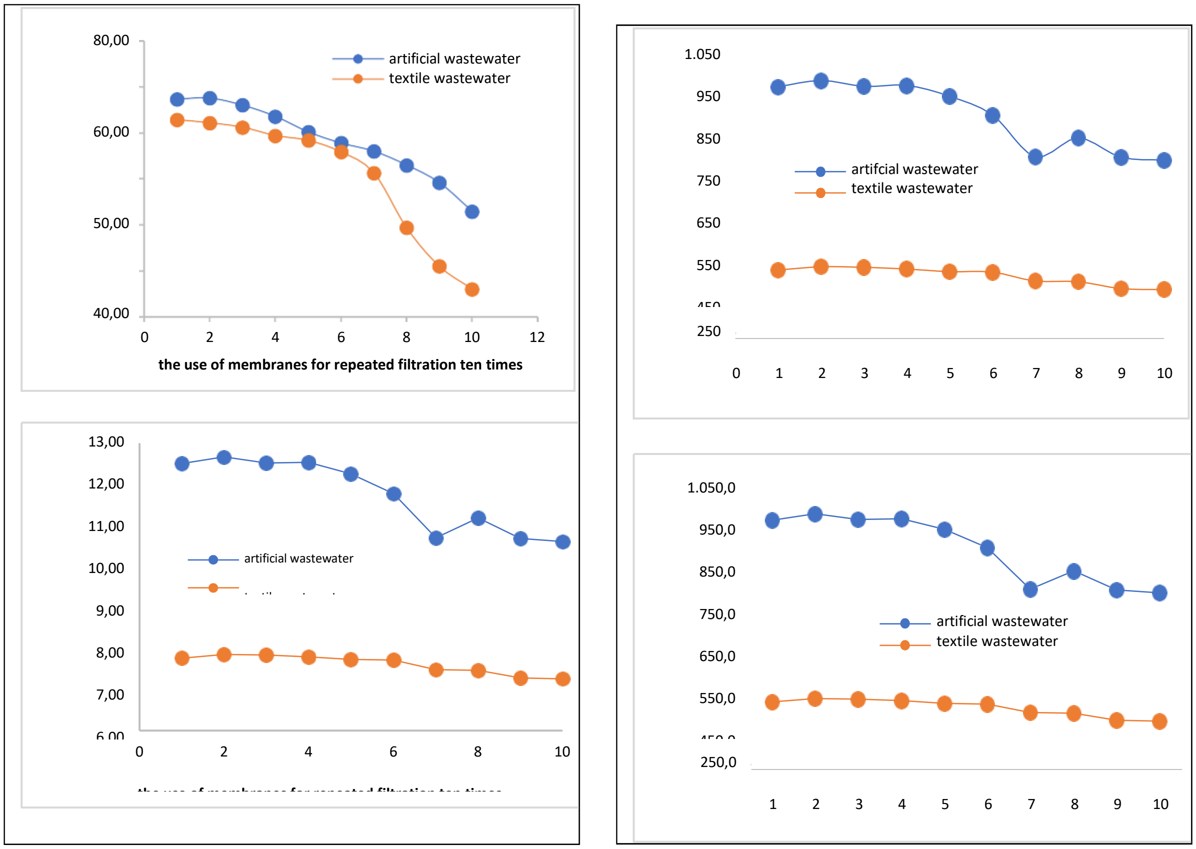


Figure 3. Profile of ten times filtration usage CS(4%)-PVA/PPO-Cu2+ membrane for artificial and textile wastewater samples based on (a) phenol rejection; (b) permeability; (c) flow rate; and (d) flux.

Figure 3a shows a graph of the decrease in phenol rejection when used 10 times. The optimum rejection obtained was 63.69% in the 1st filtration in the first batch, and stable rejection at the 1st to 7th filtration was more than 50%. The decrease in phenol rejection, one of which is caused by fouling on the membrane due to macro components in the waste with the formation of a filter cake, can affect the performance of the CS(4%)-PVA/PPO-Cu2+ membrane in addition to rejection, namely flow rate, permeability, and flux. The backwash method uses a buffer solution for filtration as an effort to reduce fouling or reactivate membrane performance. In dead-end or direct filtration, all of the feedwater passes through the membrane, resulting in nearly 100% recovery, and a small portion is periodically used for backwash in the system in the range of 2-15% [7]. Based on Figure 3a, the concentration of textile waste with a standard deviation of 0.87 indicates a low phenol rejection, so the concentration of samples from textile waste spreads the data not too wide. Thus, the CS(4%)- PVA/PPO-Cu2+ membrane performs quite well and regularly.

Figure 3b describes the correlation profile between the concentration of phenol, catechol, and quinone after the filtration process of the CS(4%)-PVA/PPO-Cu2+ membrane ten times using a batch system. The effective membrane performance is stable on the 2nd to 7th use, and the 8th to 10th use of the membrane with the lifetime of the membrane is no longer effective.

Meanwhile, the concentration of catechol was relatively decreased, which indicated that the permeate phase leading to the formation of phenol, it was indicated an increase in the concentration of phenol. In this condition, more phenol concentration was formed. Thus, the quinone concentration decreased as the phenol concentration increased because the ejected phenol would be converted to catechol and then to a quinone. The performance of the membrane is effective in the use of the 2nd to 4th membrane with PPO as a biocatalyst for the oxidation of phenol to catechol and quinone. The optimum membrane performance for the conversion of phenol to quinone is about ±40%, and the conversion of phenol to catechol is ±15%. Phenol will be oxidized enzymatically by PPO into catechol and then quinone [19]–[22].

In Fig. 3d, the flux curve profile and permeability are relatively more stable at the use of stages 2 to 6 times. There is a decrease of flux in the use of stages 7 to 10 times, which is caused by irreversible fouling or blockage of the membrane from dissolved particles in the effluent. Irreversible fouling, indicates internal fouling in the pores of the membrane and can only be removed by chemical cleaning [7]. The permeability value is the same as the flux value, because, in the filtration process, the pressure used is 1 bar, through the optimum working approach of the vacuum pump used. Ultrafiltration membranes have the ability in the pressure range of 1-5 bar which affects the effectiveness of a substance in passing through the membrane. The permeability value obtained is 300 – 1000 L/h.m2.bar, this value is still below the microfiltration membrane limit of 2000 L/h.m2.Bar and above the ultrafiltration membrane limit of 50 L/h.m2.Bar [17]. Based on the amount of flux and permeability, this CS(4%)-PVA/PPO-Cu2+ membrane is quite stable up to 6-8 times of use with a capacity of 4-5 L and the total time required is about ± 15 minutes at a pressure of 1 bar.

Textile wastewater parameter test results based on quality standards

The results of the textile wastewater parameter test based on the quality standard of the Minister of Environment Regulation No. 5 of 2014, before and after using CS(4%)-PVA/PPO-Cu2+ membrane.

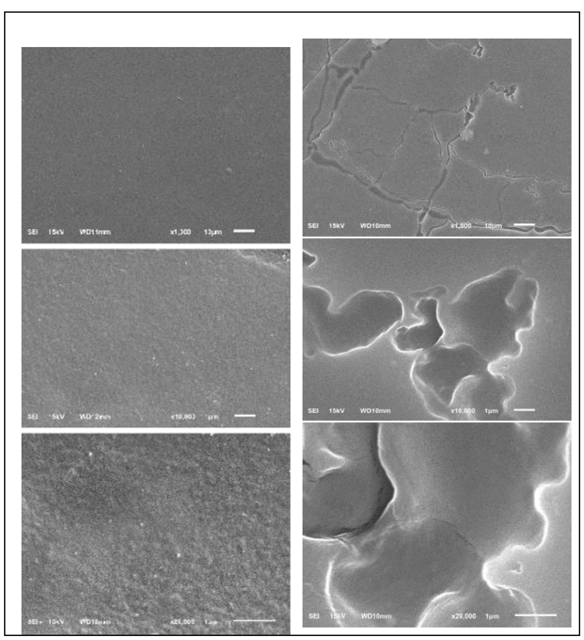
Table 3. Textile wastewater parameters before and after filtration

	Parameters	Before Filtration	After Filtration	Decreasing content (%)	Waste-water quality standard [23][23]
1	BOD5 (mg/L)	53.90	40.70	24.49	60
2	COD (mg/L)	136.00	86.40	36.47	150
3	TSS (mg/L)	47.00	6.33	86.53	50
4	phenol	0.02	0.02	41.89	0.50

SEM image for the CS(4%)PVA and CS(4%)PVA/PPO-Cu2+membranes

In Fig. 4a SEM image with 1000 x magnification; 10000 x and 20000 x for CS(4%)PVA membrane and Fig. 4b for CS(4%)PVA/PPO-Cu2+ purple eggplant membrane. The immersion of CS(4%)PVA membrane can be observed in pore opening on the CS(4%)PVA/PPO-Cu2+membrane, this is further clarified by the magnification of the measuring scale on the surface visible SEM, and the various pore sizes. At 100x magnification, the pore size of the membrane is estimated to be below 10 µm, as well as at 10,000x and 20,000x magnification, the membrane pore size is below 10 µm, and the CS(4%)PVA-PPO- Cu2+membrane is classified as microfiltration as described by Mulder [17]. SEM image of apple polypyrrole-crude membrane PPO, with PPO activity=2287 U, has been reported as filtration with a pore size of 10-20 µm [11]. The SEM-EDS image in Figure 5 describes some of the main components of the purple eggplant CS(4%)PVA/PPO-Cu2+membrane. The main chemical components are C (0.277 keV) = 79.26 %; supporting components, namely oxygen (0.525 keV) = 19.22 %. Meanwhile, the obtained chemical components such as Cu by 1.52%, because the membrane has immobilized PPO- Cu2+.membrane; (b). CS(4%)-PVA-PPO-Cu2+ membrane

a. **b.**



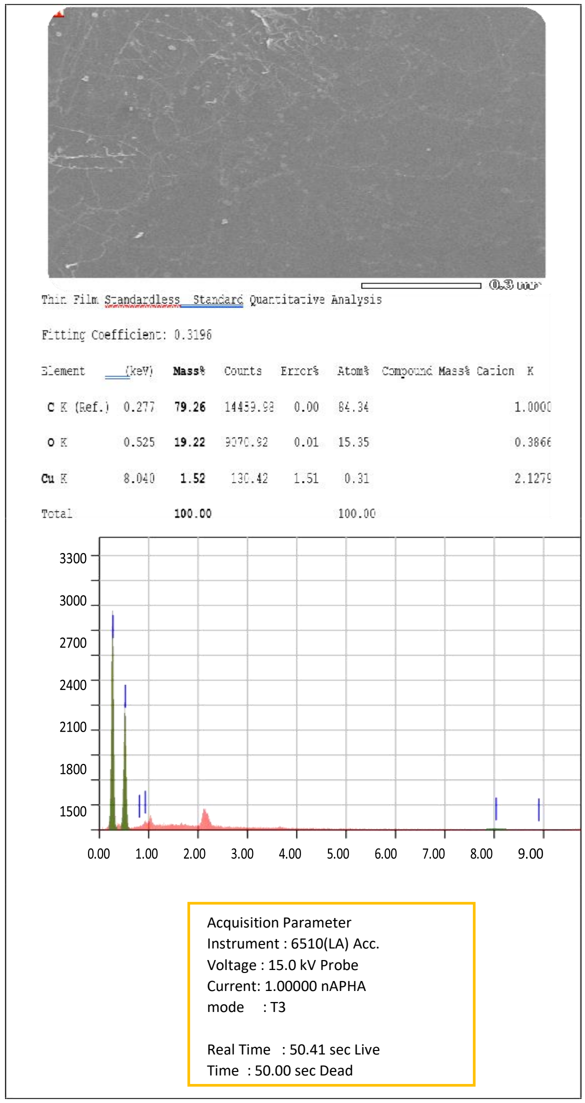


Figure 5. SEM-EDS image of CS(4%)-PVA-PPO- Cu2+ membrane

CONCLUSION

The results showed that CS(4%)-PVA/PPO-Cu2+ membrane with crude-PPO was obtained from purple eggplant extract. Furthermore, the CS(4%)-PVA/PPO- Cu2+ membrane was designed as a dead-end system filtration device, with three filtration treatments, and ten times the use of batch system membranes. The CS(4%)-PVA/PPO-Cu2+ membrane was able to remove phenol by almost 50%. The performance of the membrane on textile waste liquid samples included rejection = 63.69%; lifetime usage eight times out of ten experiments: conversion of phenol to catechol = 17.19% and conversion of phenol to quinone = 42.64%. The membrane active period is stable up to 8 uses from 10 repetitions of the batch system during the filtration process for feed volume = 4-5 L. Meanwhile, membrane operations include: flow rate =± 5 L/hour; flux= 10007.99 L/m2.h at a pressure of 1 Bar; and permeability= 300–1000 L/m2.h.Bar. This membrane can reduce COD and BOD values in textile industrial waste samples. Based on the pore size, the CS(4%)-PVA/PPO-Cu2+ membrane is classified as microfiltration in the range of 0.5 to 10 µm. The SEM- EDS image describes several main components of the CS(4%)-PVA-PPO-Cu2+ membrane with the main components being C (0.277 keV) = 79.26%; oxygen (0.525 keV) = 19.22 % . Meanwhile, the obtained chemical components such as Cu by 1.52%, because the membrane has immobilized PPO-Cu2+.

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REFERENCES

i. A. Gami, M. Y. Shukor, K. A. Khalil, F. A. Dahalan, A. Khalid, and S. A. Ahmad, "Phenol and its toxicity," Journal of Environmental Microbiology and Toxicology, vol. 2, no. 1, pp. 11–23, 2014, doi: 10.54987/jemat.v2i1.89.

ii. M. L. H. R. "Indonesia, "Perubahan 1. BAKU_MUTU_AIR_LIMBAH_menlhk_07 162019080451." pp. 1–4, 2010.

iii. V. Edalli SDVS Sangh, S. Arts, V. A. Edalli, and C. M. Kamanavalli, "REMOVAL OF PHENOLIC COMPOUNDS BY MUSHROOM POLYPHENOL OXIDASEFROM PLEUROTUS SPECIES," 2010.

iv. [Online]. Available:

v. https://www.researchgate.net/publication/236 256703

vi. N. Loncar et al., "Removal of aqueous phenol and phenol derivatives by immobilized potato polyphenol oxidase," Journal of the Serbian Chemical Society, vol. 76, no. 4, pp. 513–522, 2011, doi: 10.2298/jsc100619046l.

vii. Murniati, B. Buchari, S. Gandasasmita, Z. Nurachman, and N. Nurhanifah, "Characterization of Polyphenol Oxidase Application as Phenol Removal in Extracts of Rejected White Oyster Mushrooms (Pleurotus ostreatus)," Oriental Journal of Chemistry, vol. 34, no. 3, pp. 1457–1468, 2018, doi: 10.13005/ojc/340336.

viii. L. Heerema, D. Cakali, M. Roelands, E. Goetheer, D. Verdoes, and J. Keurentjes, "Evaluation of an integrated extraction process for in-situ phenol removal with micellar solutions of PEO-PPO-PEO block copolymers," Sep Purif Technol, vol. 74, no. 1, pp. 55–63, 2010, doi: 10.1016/j.seppur.2010.05.007.

ix. Basile, A. Cassano, and N. K. Rastogi, Advances in Membrane Technologies for Water Treatment, 1st ed. Woodhead Publishing Series in Energy, 2015.

x. Jafari Sanjari and M. Asghari, "A Review on Chitosan Utilization in Membrane Synthesis," ChemBioEng Reviews, vol. 3, no. 3, pp. 134–158, 2016, doi: 10.1002/cben.201500020.

xi. M. Sun, L. Yuan, X. Yang, and L. Shao, "Preparation and Modification of Chitosan- based Membrane," ES Materials and Manufacturing, vol. 9, pp. 40–47, Sep. 2020, doi: 10.30919/esmm5f780.

xii. J. Shao, H. Ge, and Y. Yang, "Immobilization of polyphenol oxidase on chitosan–SiO2 gel for removal of aqueous phenol," Biotechnol Lett, vol. 29, no. 6, pp. 901–905, 2007, doi: 10.1007/s10529-007-9329-2.

xiii. Murniati, B. Buchari, S. Gandasasmita, Z. Nurachman, A. Hardian, and D. Triani, "Immobilization of Crude Polyphenol Oxidase Extracts from Apples on Polypyrrole as a Membrane for Phenol Removal," Jurnal Kimia Sains dan Aplikasi, vol. 24, no. 2, pp. 62–69, Mar. 2021, doi: 10.14710/jksa.24.2.62- 69.

xiv. U. Jenderal, A. Yani, B. Buchari, and S. Gandasasmita, "Synthesis and characterization of polypyrrole polyphenol oxidase (PPy/PPO) on platinum electrode Synthesis and Characterization of Polypyrrole Membrane containing Polyphenol Oxidase View project Murniati Anceu," 2012. [Online].Available:

xv. https://www.researchgate.net/publication/282 051084

xvi. Murniati et al., "Modification of Cu2+ in polyphenol oxidase extract from purple eggplant for phenol degradation in coal wastewater treatment," IOP Conf Ser Earth Environ Sci, vol. 882, no. 1, p. 012071, 2021, doi: 10.1088/1755-1315/882/1/012071.

xvii. L. Peng et al., "Characterization of a novel polyvinyl alcohol/chitosan porous hydrogel combined with bone marrow mesenchymal stem cells and its application in articular cartilage repair," BMC Musculoskelet Disord, vol. 20, no. 1, p. 257, May 2019, doi: 10.1186/s12891-019-2644-7.

xviii. P. B. Palani et al., "Improvement of proton conductivity in nanocomposite polyvinyl alcohol (PVA)/chitosan (CS) blend membranes," RSC Adv., vol. 4, no. 106, pp. 61781–61789, 2014, doi: 10.1039/c4ra10788h.

xix. C. L. Radiman and V. Suendo, "Pengaruh Media Perendam Terhadap Permeabilitas Membran Polisulfon Dye Sensitized Solar Cell carbon based Materials for counter electrode View project Nanosilicon View project." [Online]. Available: https://www.researchgate.net/publication/277 838977

xx. U. Kragl, "Basic Principles of Membrane Technology. (2. Aufl.) VonM. Mulder. Kluwer Academic Publishers, Dordrecht, 1996. 564 S., geb. 174.00 £.—ISBN 0–7923–4247-X," Angewandte Chemie, vol. 109, no. 12, pp. 1420–1421, 1997, doi: 10.1002/ange.19971091244.

xxi. Y. Ma, F. Shi, J. Ma, M. Wu, J. Zhang, and C. Gao, "Effect of PEG additive on the morphology and performance of polysulfone ultrafiltration membranes," Desalination, vol. 272, no. 1–3, pp. 51–58, 2011, doi: 10.1016/j.desal.2010.12.054.

xxii. "Polyphenol oxidase (PPO) based biosensors for detection of phenolic compounds: A Review," Journal of Applied Biology & Biotechnology, 2017, doi: 10.7324/jabb.2017.50313.

xxiii. P. Mazzafera and S. P. Robinson, "Characterization of polyphenol oxidase in coffee," Phytochemistry, vol. 55, no. 4, pp.285–296, 2000, doi: 10.1016/s0031-

xxiv. 9422(00)00332-0.

xxv. B. Mishra, S. Gautam, and A. Sharma, "Purification and characterisation of polyphenol oxidase (PPO) from eggplant (Solanum melongena)," Food Chem, vol. 134, no. 4, pp. 1855–1861, 2012, doi: 10.1016/j.foodchem.2012.03.098.

xxvi. J. R. Whitaker, A. G. J. Voragen, and D. W. S. Wong, Handbook of food enzymology. Marcel Dekker, 2003.

xxvii. M. Lingkungan, H. Dan, and K. Republik, "Perubahan 1.

xxviii. BAKU_MUTU_AIR_LIMBAH_menlhk_07 162019080451," pp. 1–4, 2014.