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Graphene Oxide Co-deposition on Polymer Membrane for Reactive Separation in Enzyme Membrane Reactor

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Combination of enzymatic catalysis and membrane technology is advantageous in biotechnology processes. To this regard, a simple technique to immobilize the enzyme in the membrane support via reverse filtration will be applied. Alcohol dehydrogenase (ADH) (EC 1.1.1.1) was chosen as the enzyme to be immobilized on the commercial ultrafiltration membrane in this study. ADH catalyzed formaldehyde (CHOH) to methanol (CH_3OH) and simultaneously oxidized nicotinamide adenine dinucleotide (NADH) to NAD^+. An integrated, hydrophilic graphene oxide-based polymer membrane was used as a support to immobilize the enzyme. The objective of the study is to assess the performance of the biocatalytic membrane reactor when graphene oxide (GO) nanoparticles is co-deposited together with ADH enzyme within the polyethersulfone (PES) membrane in terms of its membrane permeability, hydrophilicity, enzyme loading, reaction conversion and biocatalytic productivity. The results showed that the membrane permeability increased with the addition of GO which indicating improved membrane hydrophilicity. Enzyme loading is highest for PES/GO 0.1 membrane at 79 %. The reaction conversion for all the membranes recorded between 60 % to 84 %, however the PES/GO membranes showed to be higher than the pristine membrane. The enrichment of GO improved the activity of ADH in 5 cycles. The cumulative biocatalytic productivity of PES/GO membranes in 5 consecutive cycles were higher with 455.4 μmol CH_3OH/mgADH·h and 390.5 μmol CH_3OH/mgADH·h respectively in comparison to only 359.5 μmol CH_3OH/mgADH·h for pristine membrane. The synergy between enzyme catalysis and membrane filtration is beneficial because it allows for both enzyme immobilization and simultaneous product separation.

1. Introduction

The best example for reactive separation is enzymatic membrane reactor (EMR) where biocatalytic conversion by enzymes combines with simultaneous product recovery via filtration. EMR is utilized as a tool for the enzymatic reactions of interest, as well as physical filtration in which membrane can act both as catalytic unit and/or contactor to retain enzymes inside the reactor. The membrane's porous nature allows for the reuse of enzymes, continuous operation, simple product purification, and prevention of product inhibition. It can also serve as a support for enzyme immobilization (Ismail et al., 2021). These advantages contribute to increase productivity and enhance economic viability of the process.

Graphene oxide nanoparticles (GO) is a promising material to be employed for membrane construction due to its exceptional transport capabilities, cheap manufacturing cost, incredible mechanical strength, and chemical stability (Ng et al., 2021). Graphene is a layer of graphite with a single atomic carbon, whereas graphene oxide is oxidized graphene with a monolayer of graphite oxide. GO is derived from the exfoliation of graphite oxide, is known to have a high affinity towards water molecules (Bai et al., 2019). Due to the presence of oxygen functional groups including the hydroxyl, carboxyl, carbonyl, and epoxy groups, GO and its derivatives have attracted interest for usage as nanofillers in membrane applications. It has been reported that the integration of

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GO in a hydrophobic polymer membrane can improve the wettability of the membrane and become more hydrophilic (Gholami & Mahdavi, 2018). Hydrophilic membrane will further improve membrane permeability.
In this study, the membrane fitted in EMR is co-deposited withGO to improve the hydrophilicity of the membrane. Apart from that, it is expected that the presence of GO in the membrane material would increase the absorption capacity of the membrane itself (Niedergall et al., 2014). With the subsequent enzyme immobilization, the absorbed components by the membrane absorber can be degraded into derivatives by the means of enzymatic catalysis. It is expected that the integration of GO with the membrane and subsequent enzyme immobilization would increase the performance of membrane separation. PES polymer membrane is used as the basis due to its outstanding mechanical properties and thermal stability (Al-Hinai et al., 2017). This work evaluates the effect of GO deposition together with ADH enzyme in the PES membrane in terms of its membrane permeability, hydrophilicity, enzyme loading, reaction conversion and biocatalytic productivity. ADH catalyse the reverse reaction of formaldehyde to methanol in the presence of NADH as the co-factor.

2. Methodology

2.1 Material

Formaldehyde (37 % w/w) and β-nicotinamide dinucleotide (NADH) were acquired from Sigma-Aldrich together with Alcohol Dehydrogenase (ADH) (EC 1.1.1.1) from *Saccharomyces cerevisiae* (St. Louis, MO, USA). Sodium acetate trihydrate and acetic acid, dipotassium phosphate (K_2HPO_4) and monopotassium phosphate (KH_2PO_4), Trizma-base and hydrochloric acid for buffer preparation were purchased from R&M Chemicals (Shah Alam, Malaysia). Commercial ultrafiltration PES membrane size is 30 kDa (Synder, USA). Graphite powder (MW = 12.01), potassium permanganate ($KMnO_4$), sulphuric acid ($H2_SO_4$, 95–98 % purity), sodium nitrate ($NaNO_3$, 98 %), hydrogen peroxide (H_2O_2), and hydrochloric acid (HCl, 38 %) were purchased from Merck (new Jersey, USA) and were used in the synthesis and washing of GO.

2.2 Graphene oxide synthesis

Hummer's method was used to synthesized GO and adopted from the previous work (Jamil et al., 2021). Graphite powder and $NaNO_3$ were dissolved and stirred in H_2SO_4. $KMnO_4$ was added into the beaker and stirred overnight at room temperature, before the solution was agitated for another 4 h at temperature of 15 ºC. The resulting suspension was then heated to 70 ºC for 2 h and diluted with 100 mL of DI water. H_2O_2 was then added to terminate the reaction. The solution was then washed using HCl solution and DI water for several times, centrifuged and dried for 24 h at 60-70 ºC before being purified using acetone, vacuum filtered and dried in oven at 60 °C for 6 h. Membrane images were observed using a field emission scanning electron microscope (JSM 6700F, JEOL, Japan) with acceleration voltage of 5 kV. The samples were spin-coated with gold layer (coating time: 120 s, coating current: 20 mA) before the test.

2.3 Enzyme membrane reactor set-up

PES membrane was added with GO at different concentration (0.05 and 0.10 g/L) by reverse filtration method (Luo et al., 2013). GO was previously dispersed in tris buffer, pH 7. Reverse filtration is defined by switching the support layer of PES membrane facing the feed. The membrane is fitted in a dead-end, 50 mL stirred cell (Amicon C3259, Merck Milipore, Germany). After that, ADH enzyme (3 mg) immobilization took place at enzyme concentration of 0.10 g/L and pH 7. The pressure was induced by the introduction of nitrogen gas and maintained at 2 bar during all operations (the addition of GO, enzyme immobilization and subsequent reaction steps). Model reaction is the reduction of formaldehyde to methanol by ADH with simultaneous oxidation of NADH as the cofactor, to test the performance of the biocatalytic membrane. 100 mM of formaldehyde and 100 µM of NADH was fed to the biocatalytic reactor. The conversion of formaldehyde to methanol was observed using a UV-Vis spectrophotometer (UV1280. Shimadzu, Japan) by analyzing the conversion of NADH to NAD^+ at 340 nm. One mol of NADH is converted, which is equivalent to one mol of formaldehyde produced in the reaction (Marpani, 2017).
The mass balance of enzyme immobilized on the membrane can be estimated from the following equation:

$$m_i = C_p V_p - C_r V_r - C_w V_w \quad (1)$$

The efficiency of ADH being immobilized in the membrane is expressed as loading percentage:

$$Enzyme\ loading = \frac{m_i}{m_t} \times 100 \quad (2)$$

where m_i is the amount of immobilized enzyme initially (mg), m_t is the amount of enzyme in the feed (mg), C_p is the enzyme concentration in permeate (mmol), C_r is the enzyme concentration in retentate (mmol), C_w is the enzyme concentration in rinsing residual (mmol), V_p is the permeate volume (mL), V_r is the retentate volume

(mL) and V_w is the washing residue volume (mL).The biocatalytic productivity is defined as the total amount of the product produced in the reaction divided by the amount of enzyme immobilized in the membrane multiply by the time taken for the filtration (reaction) to complete.

3. Results and discussion

3.1 Membrane profile

Figure 1 shows the SEM images of the membrane. The PES membrane consists of a 30 kDa skin layer and polypropylene support layer. The fibrous support layer of the membrane made it suitable for enzymes and GO adsorption (Figure 1c and 1d). In this study, a technique called reverse filtration is applied, where the support layer is the one used actively in the immobilization of GO and ADH and also during the biocatalytic conversion of formaldehyde to methanol.

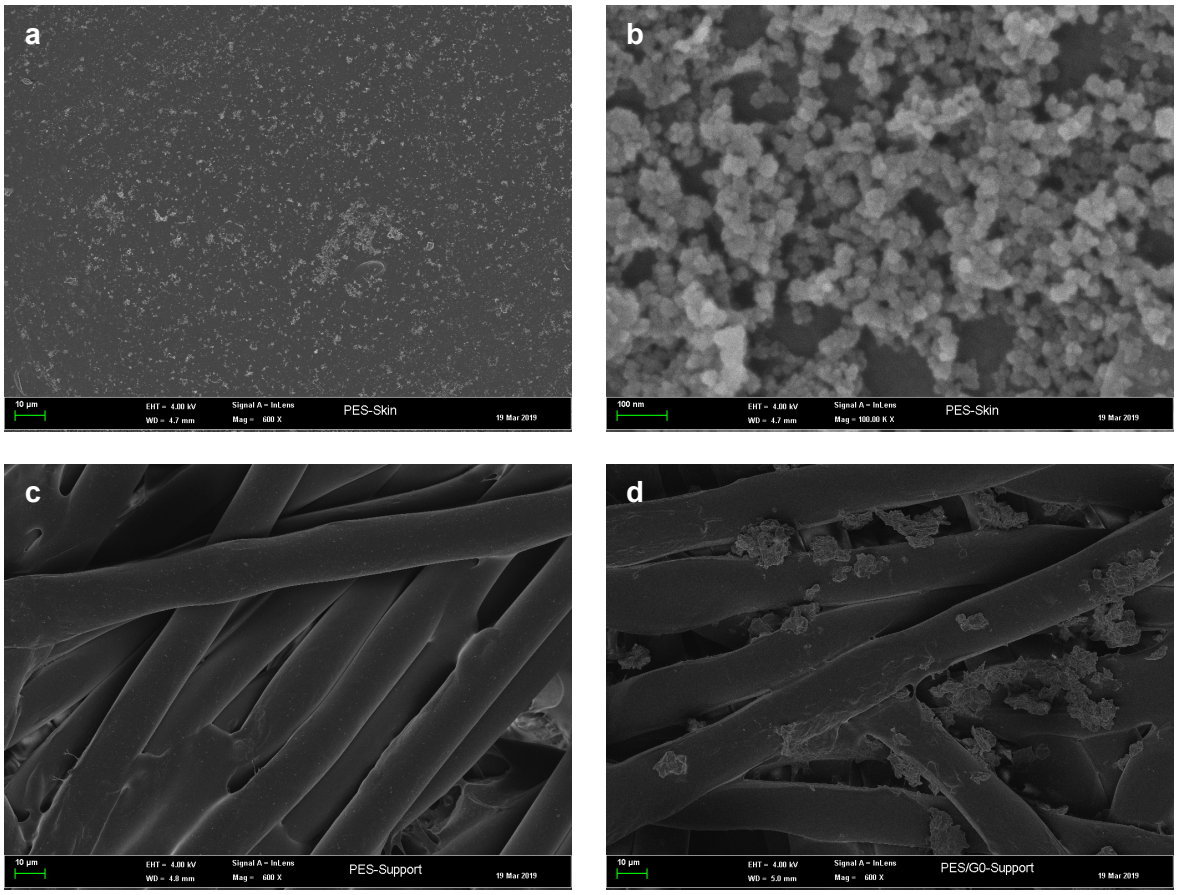

Figure 1: SEM images of PES membrane changes before and after GO co-deposition; (a) skin layer; (b) skin layer (close-up); (c) support layer; and (d) support layer after GO co-deposition.

Figure 2(a) shows the data of pure water flux of PES membrane with co-deposition of 0.05 g/L and 0.10 g/L of GO. The permeate flux passing though PES/GO membranes are higher in comparison with the pristine membrane, with permeate flux for PES/GO 0.10 membrane shows the highest (Figure 2(a)). The water contact angle value of the prepared pristine and GO co-deposited membranes is shown in Figure 2(b). In general, a smaller contact angle indicates that the membrane surface is more hydrophilic. The water contact angle of the membranes decreased significantly as the GO co-deposition percentage increased. The pristine support layer surface recorded 65.1° compared to GO/PES 0.05 which reduces to 58.1° and further declined to 55.2° showed by GO/PES 0.10 membrane (Figure 2(b)). It proves that with the addition of only 0.05 g/L GO, the hydrophilicity of the commercially available PES membrane could be increased. This explains the increment of membrane permeability exhibited in Figure 2(a) in GO co-deposited membranes compared to the pristine membrane. Hydrophilicity is important to sustain enzyme stability in its microenvironment when immobilized in the membrane (Marpani et al., 2015).

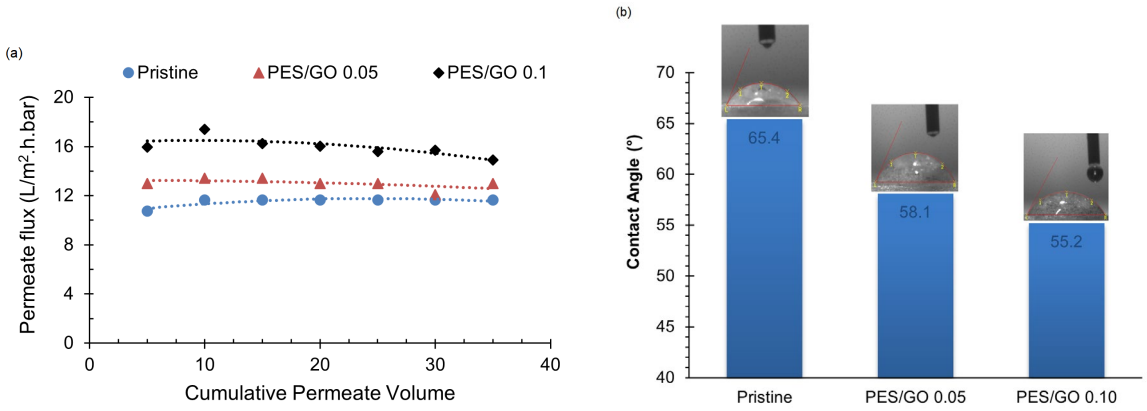

Figure 2: (a) Pure water flux through pristine PES membrane and membrane with GO added and (b) contact angle on the surface of the support layer of PES membrane.

3.2 Enzyme loading and membrane fouling

Enzyme loading for PES membrane with deposition of 0.10 g/L GO results in higher enzyme loading with 79 % (Table 1). The co-deposition of GO can enhance the enzymes adsorption capacity of the membrane and hence decreases enzyme leakage (Zhang et al., 2018). Higher GO concentration deposition may prepare more adsorption side for the attachment of enzyme functional groups (Srere & Uyeda, 1976) to hydroxyl groups which is abundant in GO molecular structure (Fuzil et al., 2022) Figure 3 shows the plotting of membrane flux during enzyme immobilization into Hermia's model. The model will predict the placement of enzymes on the membrane pores. From the model, intermediate fouling was predicted with R^2 value equals to 1. A single enzyme may aggregate with other particles during intermediate blocking to form multiple layers on the membrane surface, hence increasing the cake's thickness in the process (Rahman et al., 2021). It is worth noting that the standard blocking showed R^2 value almost 1 with 0.9963 and 0.9965. Hence, it also predicted that the enzyme adsorbed at the wall of the membrane's pores as well.

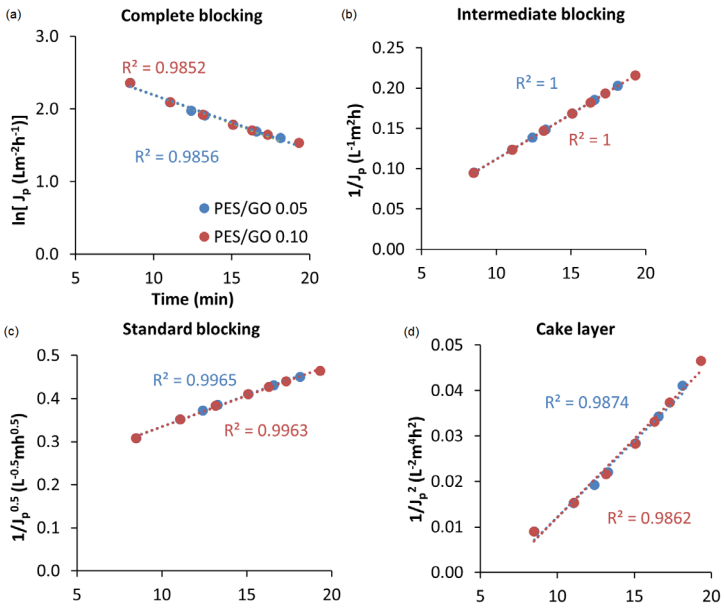

Figure 3: Membrane fouling type using Hermia's model (a) complete blocking; (b) intermediate blocking; (c) standard blocking and (d) cake layer.

Table 1: Percentage of enzyme immobilized on the PES membrane support layer.

Membrane type	ADH (mg)				Immobilized Enzyme (%)
	Feed	Permeate	Retentate	Washing	
Pristine	3.0	0.6950	0.0437	0.1330	65.94
PES/GO 0.05	3.0	0.6272	0.0216	0.2217	70.98
PES/GO 0.10	3.0	0.5030	0.0241	0.1011	79.06

3.3 Reactor performance

The performance of the biocatalytic membrane was tested by using model reaction converting formaldehyde to methanol utilizing NADH as the co-factor. The control experiment is the pristine membrane with the enzyme immobilized in the membrane without the GO co-deposition. From Figure 4, the percentage conversion of formaldehyde to methanol is higher in PES/GO membranes compared to the pristine membrane. The highest conversion at 84 % was recorded by the PES membrane with co-deposition of 0.10 g/L GO. Nevertheless, the trend of methanol conversion and biocatalytic productivity are decreasing with consecutive cycles. The PES/GO 0.10 and PES/GO 0.05 membranes recorded a stable conversion and reached 68.3 % and 57.3 %, even after 5 cycles, whereas the conversion for pristine membrane drop to the lowest of only 10.5 %. The enzymes in the PES/GO membranes showed higher loading (Table 1) due to increased adsorption capacity which subsequently have high tendency to adsorb not only the enzyme and the substrate, but also the buffer (water) causing increased enzyme activity due to hydrophilic microenvironment. Biocatalytic productivity is defined as the ratio of amount of product produced per amount of enzyme used in the reactor, primarily used to evaluate the efficiency of immobilized enzymes. In this study, the factor of reaction time is taken into consideration to further evaluates the amount of methanol produced for 5 consecutive cycles. The pristine membrane shows the highest biocatalytic productivity in the first cycle compared to the membranes with GO. The conversion of pristine membrane is only 60 %, much less than the PES/GO membranes. However, the time taken to complete the reaction in the pristine membrane was 50% faster in pristine membrane compared to PES/GO membranes. The pristine membrane has no obstruction of GO, hence shorter reaction time but enzyme is less stable in the long run (obvious conversion decrease after the first cycle (Figure 4)). After the second cycle, The PES/GO membranes showed a much higher conversion compared to the pristine membrane, which record a higher biocatalytic productivity in that case. Even though pristine membrane has shorter reaction time, cumulative biocatalytic productivity of PES/GO membranes in 5 consecutive cycles were higher with 455.4 µmol CH^3OH/mgADH·h and 390.5 µmol CH_3OH/mgADH·h in comparison to only 359.5 µmol CH_3OH/mgADH·h for pristine membrane.

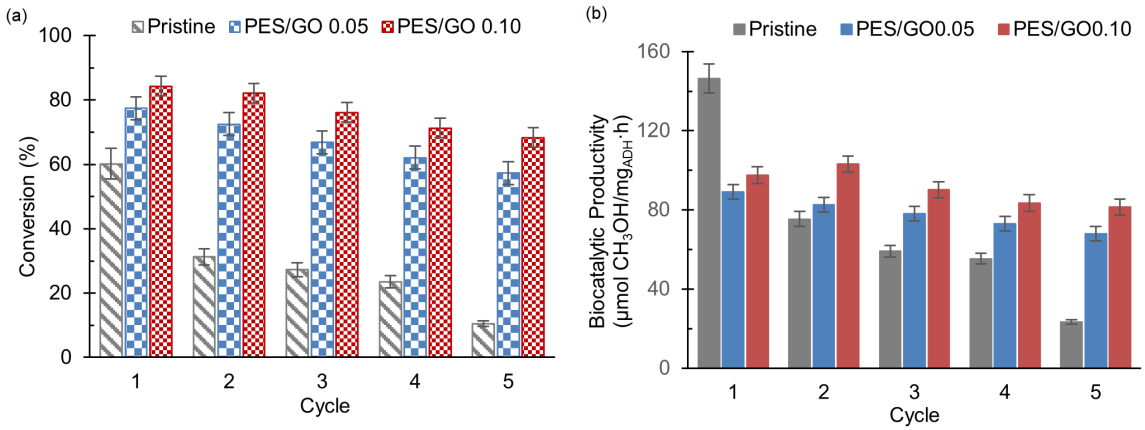

Figure 4: (a) Percentage conversion of formaldehyde to methanol for 5 consecutive cycles and (b) biocatalytic productivity of ADH enzyme in the enzymatic membrane reactor.

4. Conclusions

It can be concluded that the addition of GO by reverse filtration on the surface of the membrane support can enhance the permeability and hydrophilicity of the commercially available PES. The enzyme loading also increase with PES/GO membranes compared to the pristine membrane. Hermia's model predicted intermediate fouling mechanism was observed. The co-deposition of GO in PES membrane has shown to increase the activity of enzymes. It was proven with up to 84 % of methanol is converted from formaldehyde in PES/GO 0.10

membrane. After 5 consecutive cycles, the biocatalytic PES/GO membrane reactor still able to convert more than 50 % of formaldehyde with 68.3 % and 57.3 % for PES/GO 0.01 and PES/GO 0.05. Cumulative biocatalytic productivity for PES/GO membranes conclusively became the higher end after 5 consecutive cycles which shows that the co-deposition of GO could enhance the enzyme activity.

Nomenclature

m_i – amount of immobilized enzyme initially, mg
m_t – amount of enzyme in the feed, mg
C_p – permeate enzyme concentration, mmol
C_r – retentate enzyme concentration, mmol
C_w – rinsing residual enzyme concentration, mmol
V_p – permeate volume, mL
V_r – retentate volume, mL
V_w – washing residue volume, mL
J_p – permeate flux, $L/m^2 \cdot h \cdot bar$

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