

## Assessing the Effect of PVP of Various Molecular Weight (MW) in PES-Ag Membranes: Antimicrobial Study Using *E. Coli*

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### Abstract

*New polymeric silver (Ag) composites were prepared using polyethersulfone (PES) as polymer material, N-Methyl-2-pyrrolidone (NMP) as solvent and polyvinylpyrrolidone (PVP) of various molecular weight (MW) as organic fillers. Silver nitrate (AgNO<sub>3</sub>) was added by various loading in order to impart an antibacterial property to the composites. The miscibility of PES/Ag/fillers composites were characterized using TGA and any changes in chemical bonding were characterized using ATR-FTIR techniques. The XPS results showed high intensity in E<sub>b</sub>(Ag<sub>3d</sub>) range of 378.1-378.5 and 372.2-372.5 for the highest Ag loading with PVP with the highest MW. This phenomenon had altered the hydrophilicity property of composites. In this study, composites have undergone a bacteria rejection test from *E.coli* suspension and a domestic waste sample. Composites with highest loading of silver and highest MW of PVP (PES-Ag<sup>2</sup>P360) was observed for significantly high bacteria rejection, log reduction value (LRV~3). Therefore, PES-silver nanocomposites is a potential membrane for bacteria removal, disinfection and maybe effective for overcoming biofouling problems.*

**Keywords:** polyethersulfone-silver; composites; polyvinylpyrrolidone; antibacterial

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## 1. INTRODUCTION

Recently, combination of polymeric materials with silver has attracted increased attention in the area of antibacterial membrane [1-5]. The high antibacterial activity with very low loading and the potential to obtain smaller size in addition to better distribution of silver particle are the essential properties of these nanocomposites. However, the long-term stable and efficient operation of these nanocomposites requires that the surfaces remain completely silver-filled over the prolonged periods of operational time. Therefore, quite a number of researches have been carried out in order to improve the silver particle distribution and its stability. Cellulose acetate (CA), chitosan, polyacrylonitrile (PAN) and polysulfone (PSf) are the most popular polymeric materials studied [2,4-6]. However, since CA, chitosan and PAN limitations such as cannot be dissolved in common organic solvent and considered expensive material, polyethersulfone (PES) is chosen in this study. In addition, PES is soluble in the organic solvents, easy-handling polymeric material, and resistance to chemical attack and has reasonable hydrophilicity [7-9].

The role of polyvinylpyrrolidone (PVP) in silver capping has received considerable attention due to the chemical structure of PVP that contains N atom which can protect the silver particles from growing and agglomerating [10]. Slistan *et al.* discovered the interaction of silver atom with carbonyl groups of ethylene glycol (EG) when PVP-Ag was pre-treated with EG and led to silver particles size reduction, hence altered the original properties of colloidal silver [11]. Zhang *et al.* suggested the mechanism on silver protection by PVP through steric effects that prohibited silver particles from growing and agglomerating [12]. To the best of our knowledge, the effects of silver loading and different molecular weight (MW) of PVP will be presented in this study and the correlation to the antibacterial behavior of the PES-silver nanocomposites fabricated was also offered.

PES-silver nanocomposites is a potential membrane for bacteria removal, disinfection and maybe effective for overcoming biofouling problems. Therefore, composites loaded with evenly distributed silver particles were observed for significantly high bacteria rejection in terms of log reduction value.

## 2. EXPERIMENTAL

### 2.1 Samples Preparation

Nanocomposites were prepared from PES (PES, RADEL A-300) supplied by Amoco Chemicals, NMP (99.5%) from Merck and AgNO<sub>3</sub> (analytical grade) at appropriatory composition from Fluka. PVP at various average molecular weights (10,000, 40,000 and 360,000) were purchased from Fluka while distilled water was used as a coagulation medium. Microbiology agar from Merck was used in preparing plates for antibacterial test. PES was dried in an oven at 60 °C for overnight before being used remove moisture. Measurement was carried out under nitrogen atmosphere in the temperature range of 50-750 °C with the heating/cooling were done at the rate of 20 °C/min. TGA measures weight changes as a function of temperature, while other organic chemicals were obtained in reagent grade purities and used as received.

Casting solution generally consisted of PES (polymer), NMP (less volatile solvent), AgNO<sub>3</sub> (antibacterial agent) and PVP (organic filler). Detailed composition of the series was listed in Table 1. PES dissolved in NMP was prepared as a control. In the nanocomposite preparation, AgNO<sub>3</sub> and PVP were first agitated in NMP. Then, PES were added slowly and continuously agitated for at least 24 h to obtain homogeneity. Dope solution was cast on a glass plate with a casting knife for gap setting of 100 mm at an appropriate casting shear. Casting was carried out at ambient atmosphere (30 °C and 84% relative humidity). Then, glass plate with the thin-film of nanocomposites were immersed in an aqueous bath at 28 °C and remained there for 1 day. Finally, the washed nanocomposites were then dried at room temperature for 1 day.

**Table 1:** Composition of dope solution used in this study

	PES	PES- Ag <sup>0.5</sup>	PES- Ag <sup>2</sup>	PES- Ag <sup>0.5</sup> P10	PES- Ag <sup>0.5</sup> P40	PES- Ag <sup>0.5</sup> P360	PES- Ag <sup>2</sup> P360
PES	15	15	15	15	15	15	15
NMP	85	84.5	83	84	84	84	81
AgNO <sub>3</sub>	-	0.5	2	0.5	0.5	0.5	2
PVP-P10*	-	-	-	0.5	-	-	-
PVP-P40*	-	-	-	-	0.5	-	-
PVP-P360*	-	-	-	-	-	0.5	2

\* molecular weight (KDa) : P10 : 10, P40 : 40, P360 : 360

## 2.2 Characterization of nanocomposites membrane

### *Miscibility analysis*

FTIR-ATR is an innovative, non-destructive technique widely used to monitor the diffusion behavior within polymers. The ATR technique enables the original FTIR instrument to monitor species in-situ while providing additional chemical information on any changes that may be occurring during the transport process. FTIR is also a powerful instrument to reveal information about bonding vibration states (stretching and bending) of a molecule. In this study, the IR-spectra were recorded on Thermo Nicolet 5700 ATR-FTIR spectrophotometer, which is supplied by Thermo Nicolet Corporation & Spectra Tech, USA. Nanocomposites samples were cut (5 cm<sup>2</sup>) and dried at 60 °C overnight before analysis in order to eliminate the moisture content that may adhere on spectrum. Samples were then mounted at a position with skin surface facing the IR beam.

All PES control and six composite membranes were analyzed using Mettler Toledo thermogravimetry analyzer (TGA/SDTA851e). Thermogravimetric spectra reveal information on composites thermal stability and decomposition trend of any components in the composites system (if any). The wettability of the modified and unmodified composite membranes was determined by contact angle (CA) measurement. CA measurements were carried out by applying the sessile drop method at room temperature using a CA goniometer (IMC-159D) supplied by IMOTO Machinery Co. Ltd. The CA experiment was carried out using 2×2 cm<sup>2</sup> piece of composite membrane. A water drop of 5 µl was put on the thin film and the CA was observed within 10s. The experiment was repeated three times and the average with standard deviation values were recorded to achieve a good statistics.

### *X-Ray Photoelectron Spectroscopy*

Composite membrane surfaces were analyzed using X-Ray Photoelectron Spectrophotometer, JEOL JPS-9000SX with MgK<sub>α</sub> (1253.6 eV) as a radiation source. Samples with the area of 1×1 cm<sup>2</sup> were taken from random positions then mounted using double sided adhesive tape. For a wide-scan, photoelectron peak for C 1s was observed at binding energy (BE) 286.8 eV. All narrow scan spectra from each element of interest were calibrated with the corresponding C-1s as the standard for the charging effects correction.

### *Antimicrobial Study*

In this study, filtration experiments were done on *E.coli* suspension with the standard procedure adopted from Basri *et al.* [13]. Prior to the experiment, *E.coli* of optical density at λ = 600nm (OD<sub>600</sub>) 0.3 was used in the test. This *E.coli* suspension was filtrated through the fabricated membranes effective area 13.2 cm<sup>2</sup> using vacuum filtration cell set-up. For each type of membrane, three replicates of feed and permeate were collected. Inhibition zone analysis was carried out after incubation at 37 °C overnight.

Further performance test was carried out on domestic waste. It is noted that domestic waste do not contain only *E.coli* but also many other types of bacteria. However, discussion will

only be made on the basis of the membrane performance to reject the bacteria without classifying the type or nature of those bacteria. Samples were loaded at  $\sim 0$  °C to maintain the number of microbes originally located in the sampling spot. Removal of bacteria from domestic waste by filtration was also performed in a similar manner. However, 1 mL of feed and permeate were inoculated directly (without dilution) on agar plate and enumerated after incubation at 37 °C overnight. For each type of membrane, enumerations was carried out on at least three replicates of feed and permeate and further characterized for membrane retention efficiency using the log reduction value (LRV) according to the following relationship:

$$\text{LRV} = \log_{10} \frac{N_0(\text{CFU/mL})}{N_1(\text{CFU/mL})} \times 100\% \quad (1)$$

where  $N_0$  is the average surface bacterial load determined from the feed plates and  $N_1$  is the number of bacteria passing through prepared membranes.

### 3. RESULTS AND DISCUSSION

#### 3.1 Miscibility analyses – using TGA and ATR-FTIR

TGA curves in Figure 1 shows the thermal decomposition of PES, PES-Ag and PES-Ag-PVP (PES-Ag-P10/P40/P360) which occurred in a single step correspond to the compatibility of PES and PVP. Obviously PES degrades as early as 50 °C while PES-Ag degradation starts at 440 °C. By incorporating only 0.5 wt% of Ag, thermal stability of PES membranes seems significantly improved, in agreement with Song *et al.* [14] who reported on improved thermal stability of PCMM (phase change material microcapsules) after silver incorporation. However after adding PVP into PES-Ag membranes, degradation starts at lower temperatures such as 408, 395 and 350 °C for PES-Ag<sup>0.5</sup>P10, PES-Ag<sup>0.5</sup>P40 and PES-Ag<sup>0.5</sup>P360, respectively. This is because PVP played a role as a pore-former, contributed to pore enlargement and increase in membrane porosity, hence made the structure easier to degrade. This is in accordance with the report of Xu *et al.*, Han and Nam and Yoo *et al.* [15-17]. However, it seems that the molecular weight of PVP has some effect on the thermal stability, since PES-Ag<sup>0.5</sup>P360 degrades at the lowest temperature, 350 °C. It should also be noted that the thermal degradation pattern of PES-Ag<sup>0.5</sup> and PES-Ag<sup>0.5</sup>PVP (P10/P40/P360) membranes are quite similar.

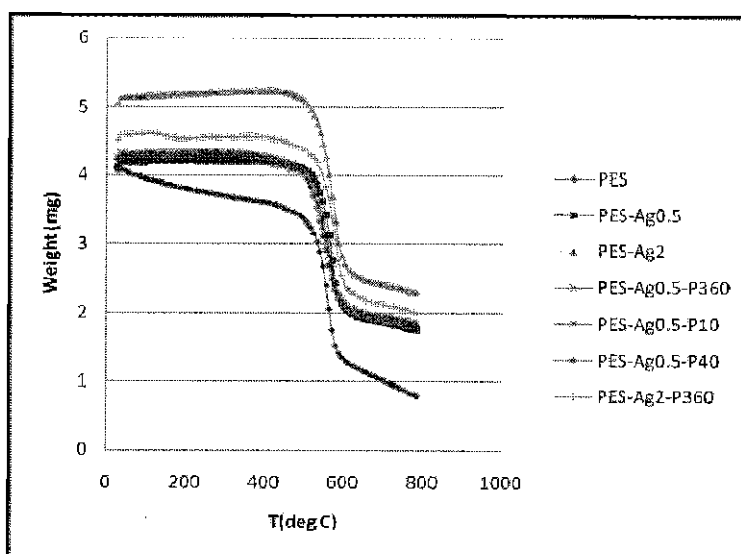
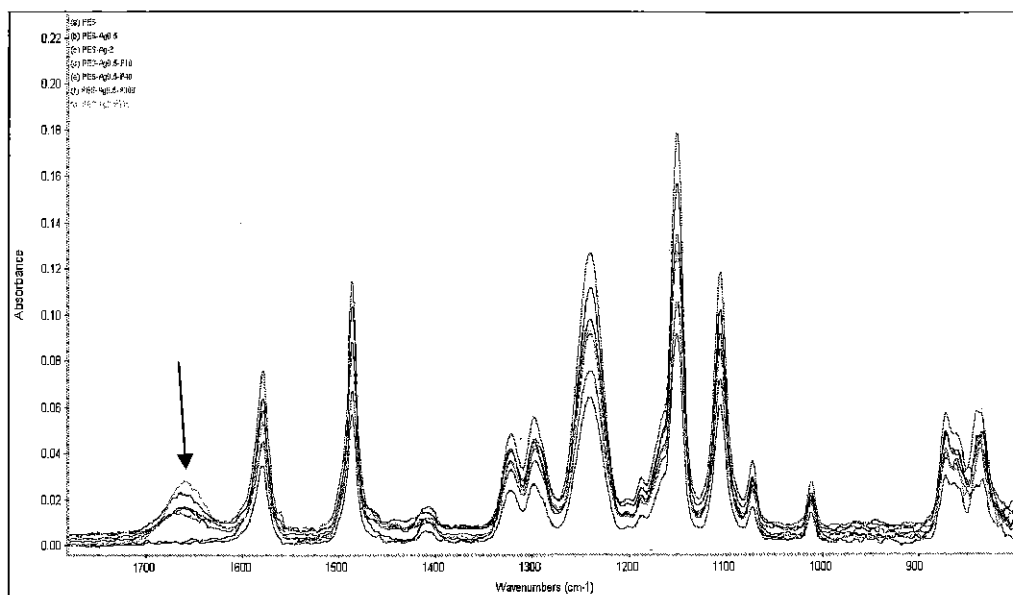


Figure 1. Thermal stability of PES-based membranes on the weight basis



**Figure 2.** FTIR Spectra of PES-based membranes

To investigate the interaction between Ag-PVP, PVP-PES and PES-Ag, ATR-FTIR spectra of the top surface on membranes are measured and shown in Figure 2. It was found that the entire IR spectra showed no apparent difference in the range of 1200-1600  $\text{cm}^{-1}$ . The spectra showed no difference between PES and PES-Ag which implied that Ag does not form chemical bonds with PES, agreed to the results obtained by Taurozzi for polysulfone (PSf-Ag) [3]. The only difference was at the absorption band of 1666  $\text{cm}^{-1}$ , the intensity of which is much greater for membranes (a), (b) and (c). This is natural since the peak is attributed to amide carbonyl group from PVP [10].

### 3.3 Contact angle (CA)

The static contact angle membrane was measured using a contact angle analysis system using CA goniometer, IMC-159D supplied by IMOTO Machinery Co. Ltd. The measurement was carried out by dropping 5  $\mu\text{l}$  of water on membrane with  $2 \times 2 \text{ cm}^2$  dimension and observed within 10 s. To minimize experimental error, the measurement was repeated ten times to obtain the average and standard deviation. Table 2 displayed CA values of prepared membranes. From the CA values, it was clearly seen that the addition of Ag has improved the membrane hydrophilicity in which the value decreased by 6 and 15% with Ag concentration of 0.5 (PES-Ag<sup>0.5</sup>) and 2 wt.% (PES-Ag<sup>2</sup>) respectively. This phenomenon could be attributed to the presence of Ag particles that has lowered the surface tension of pristine PES [18], hence water can easily spread on membrane surfaces.

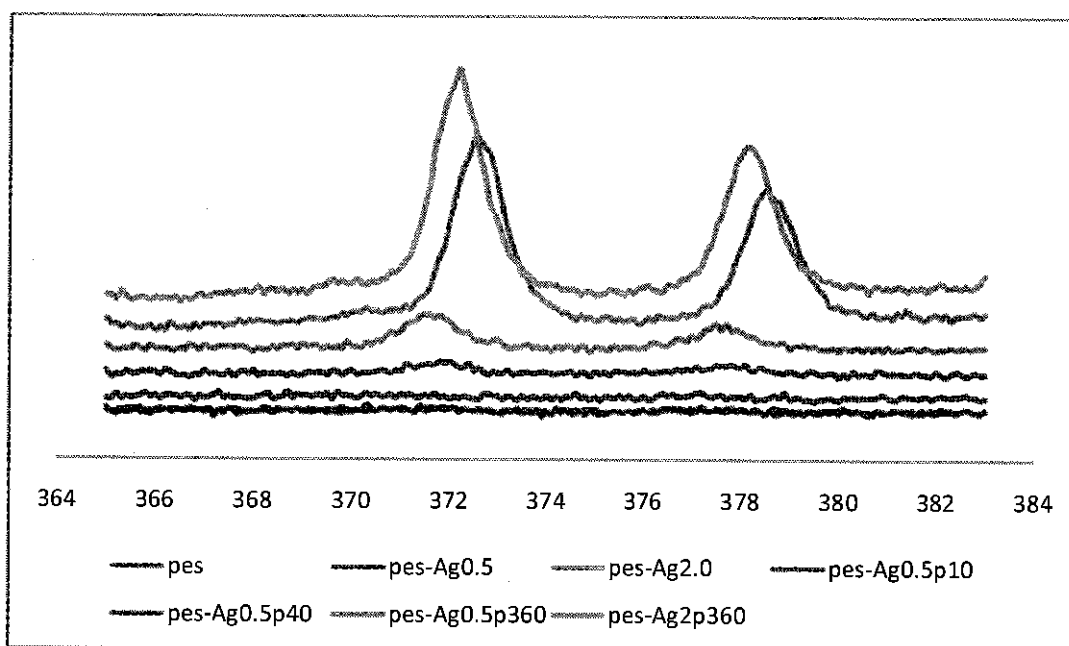
**Table 2:** Contact angle values for prepared membranes

Membrane	Contact angle $\theta$ ( $^\circ$ )
PES	$59.85 \pm 1.35$
PES-Ag <sup>0.5</sup>	$56.22 \pm 0.53$
PES-Ag <sup>0.5</sup> P10	$46.20 \pm 0.94$
PES-Ag <sup>0.5</sup> P40	$40.29 \pm 2.35$
PES-Ag <sup>0.5</sup> P360	$55.54 \pm 1.0$
PES-Ag <sup>2</sup>	$50.97 \pm 1.52$
PES-Ag <sup>2</sup> P360	$47.85 \pm 1.8$

The addition of PVP in PES-Ag<sup>0.5</sup>P10 and PES-Ag<sup>0.5</sup>P40 membrane has altered the membrane wettability to become more hydrophilic attributed to the role of PVP as water-soluble polymer to induce swelling in the membrane [19,20]. However, the contact angle value for PES-Ag<sup>0.5</sup>P360 was slightly higher ( $55.54 \pm 1^\circ$ ) and this was most probably due to the presence of Ag particles with oxidation number 0; which were highly entrapped hence induced hydrophobicity to membrane surfaces. As Ag particle is hydrophobic and weakly preferential to water molecule in nature [21], therefore the higher the MW of PVP used in PES membrane, the more Ag is entrapped which in turn increased the hydrophobicity of the membrane.

### 3.4 XPS

Figure 3 depicts the XPS spectra of each prepared membrane surface. The XPS analysis revealed significant peaks of Ag on PES-Ag membranes prepared from 2wt.% Ag and PVP of MW 360, 000. Generally, metallic silver, Ag 3d peaks are located centered at 367.9 eV (Ag 3d<sub>5/2</sub>) and 373.9 eV (Ag 3d<sub>3/2</sub>) with a spin energy separation, (the symbol,  $\Delta E$ ) of 6.0 eV [22,23]. Figure 3 depicts stacked spectra of Ag-3d which reveals that PES-Ag<sup>2</sup>P360 exhibited the highest intensity (a.u) due to the highest concentration of Ag detected on the membrane surface. As can be seen, all peaks excluding PES satisfied the spin energy separation of 6.0 eV which confirmed the successful incorporation of Ag into the membrane. However, for membranes with low concentration of Ag which are PES-Ag<sup>0.5</sup>P10, PES-Ag<sup>0.5</sup>P40 and PES-Ag<sup>0.5</sup>P360, the photoelectron peaks of Ag-3d<sup>3/2</sup> and Ag-3d<sup>5/2</sup> are low in intensity.

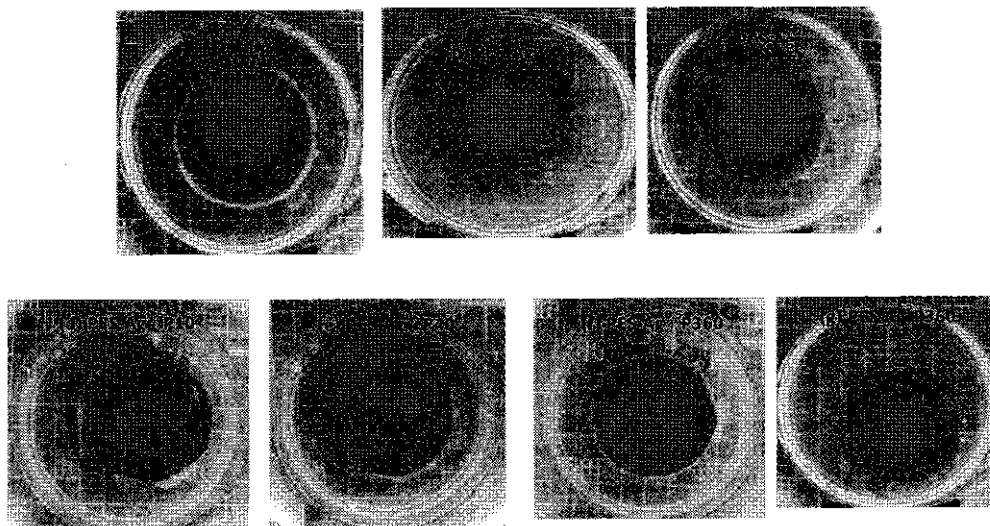


**Figure 3.** Raw XPS-spectra of Ag-3d<sub>3/2</sub> and <sub>5/2</sub> showing the intensified peak of PES-Ag membranes with PVP MW 360,000 Da

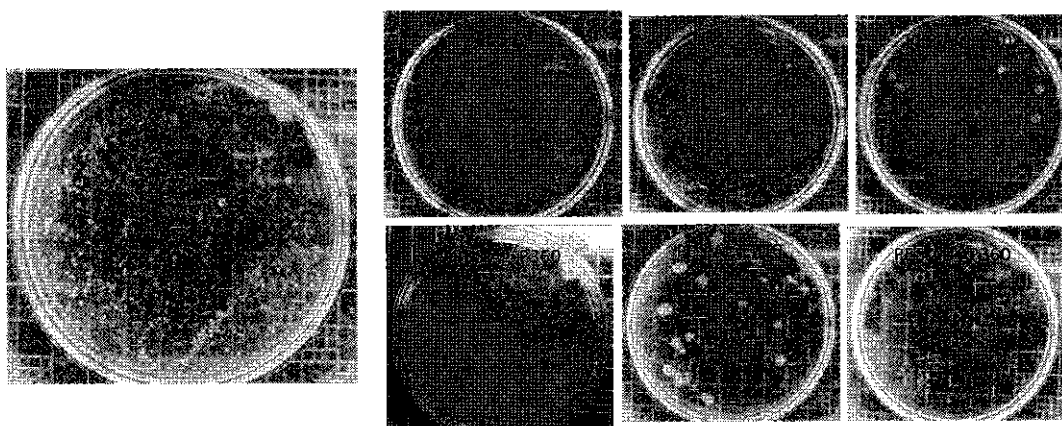
### 3.5 Antibacterial results

The results of previous disk diffusion tests on Ag-loaded membrane reported by Basri *et al.* [13] showed that at 0.5 wt.% of Ag, membranes exhibited bactericidal activity against gram-negative bacteria, *Escherichia coli* (*E.coli*) and gram-positive bacteria, *Staphylococcus aureus* (*S.aureus*) with PES-Ag<sup>0.5</sup>P360 performed the largest inhibition zone. In this study, all types of membrane were filtrated with 50 mL of *E.coli* suspension using a vacuum flask filtration set-up.

Figure 4 shows that plates numbered PES-Ag<sup>2</sup> and PES-Ag<sup>2</sup>P360 exhibited almost 100 % growth inhibition due to the release of higher dosage of diffusible inhibitory compounds from silver particles into the surrounding medium [24]. This observation is in line with the results obtained from EDX and XPS which revealed the high Ag-concentration present in both membrane. The other reason for similar performance in the antibacterial activity was the even distribution of Ag-particles possessed by both membranes (see Fig. 4). Therefore, it can be concluded that PVP of the highest MW (i.e. 360,000) has resulted in successful Ag-capping which in turn increased the concentration on membrane surfaces and further enhanced the antibacterial activity by inhibiting the *E.coli* growth.



**Figure 4.** Results of *E.coli* growth in an inhibition test



**Figure 5.** The antibacterial results of domestic waste

Filtration of domestic waste has been carried out on prepared membranes. The feed and permeates have been inoculated on agar plates and undergone incubation. Figure 5 shows bacteria grown on agar plate after 24 h incubation at 37 °C. It was observed that PES-Ag<sup>2</sup> and PES-Ag<sup>2</sup>P360 revealed the minimum bacterial activity due to bacteria-killing mechanism by Ag ion.

Therefore it can be concluded that the effect of PVP at various MW to bactericidal activity was not significant whereas the performance of PES-Ag<sup>2</sup> and PES-Ag<sup>2</sup>P360 were undoubtedly efficient with the LRV values of 1.16 (93.1 % removal) and 1.20 (93.7% removal) respectively. This could be due to the low-loading of Ag in the membrane matrix which has limited the inhibition rate. In general, Ag particles attacked bacteria from the cell membrane, disturbed its function and resulted in cell death. It is in good agreement with previous studies that the efficiency of the antibacterial activity relies on the amount and distribution of Ag particles on the membrane surfaces.

#### 4.0 CONCLUSION

Antibacterial PES-Ag membranes were successfully fabricated with PVP as dispersant in the casting solution. The effects of Ag-loading and PVP MW on the thermal properties and contact angle were investigated. Results revealed that membranes' contact angle decreased when Ag-loading was increased mainly due to hydrophilicity improvement. It is also observed in the study that the higher concentration of PVP, the more hydrophilic the membrane. From the XPS analysis, the PES-Ag membrane with 2.0 wt.% of Ag and PVP MW 360,000 displayed high Ag-intensity, thus resulted in high activity in the antibacterial test against *E.coli*. Therefore it can be concluded that the presence of PVP of MW 360,000 in the PES-Ag membrane had significantly improved membrane performances in the antibacterial activity.

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