1. Introduction:
The world, in general, and developing countries in particular, are defying water scarcity as a result of population growth, rural-urban migration, and a lack of ambient sheath [1]. Without pretreatment, sewage drop-off continuously contaminates water [2, 3]. To treat wastewater, various systems are used, but the membrane bioreactor (MBR) is widely used due to its ablated impression and excellent outflow [4, 5, 6]. However, membrane biofouling prevents MBRs from being used widely [7]. It may cause decreased infiltration and increased transmembrane pressure when manipulated [8].

The term biofouling is derived from the heat swapper technique, which involves the aggregation of unwanted substances [9, 10]. Biofouling is the most persistent and irreversible type of fouling [11, 12]. The production of extracellular polymeric substances (EPS) by microorganisms results in the formation of a morphological framework of biofilm that clutches the microorganisms and ensures their agglomeration [13, 14]. Harsh conditions do not

Abstract
Biofouling is one of the paramount complications in membrane filtration systems, which are used to purify wastewater. It increases the price tag on processes while decreasing membrane permeability and period efficiency. In the study, mycologically derived AgNPs were tested using the strong antibacterial properties of silver nanoparticles (AgNPs). Development of polysulfone membrane (Psf) infused with AgNPs for wastewater treatment, having antibacterial and antifouling properties was included in the study. Several water-drainage microbial insulates, e.g, Enterobacter aerogenes, Escherichia coli, Klebsiella pneumonia, Salmonella choleraesuis, Shigella sonnei, Pseudomonas aeruginosa, and Staphylococcus aureus appeared to be restrained against AgNPs of concentration 50-500 ppm. The AgNPs-impregnated Psf ultrafiltration membranes reduced the bacterial count (cfu/cm2) on the 7th, 21st, 30th and 45th day of experimentation in contrast to standard Psf membranes. The plain Psf-membrane CFU of 118 and 308 cfu/cm2 at the 30th and 45th day while that of the Ag-Psf membrane was 20 and 36 cfu/cm2. The efficacy of this anti-biofouling agent was demonstrated using FTIR and scanning electron microscopy (SEM) characterization techniques. In FTIR, in addition to the typical highs of the Psf layer, a new top at 687 cm-1 with a -CH=CH- pattern in the cis transfiguration was also present, demonstrating the specific degradation of the membrane due to biofouling. The SEM images indicated that AgNPs exerted to prevent biofouling; thus, AgNPs proved to be a useful solution for limiting biofilm expansion and increasing membrane life.

Keywords: Anti-biofouling, Polysulfone Membrane, Silver Nanoparticles, Wastewater Treatment

Fabrication of Anti-biofouling and Antibacterial Polysulfone Membrane Via Impregnation of Silver Nanoparticles

M. Ayub1, S.Z.H. Naqvi1,2, W. Ahmad1, M.M. Arif1, K.A. Saqib1, A. Hameed1, N. Ali2, N. Ahmad2

Submitted: 17/03/2022, Accepted: 17/02/2023, Online: 22/02/2023

1Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore, Lahore, Pakistan.
2Department of Microbiology, Quaid-i-Azam University, Islamabad, Pakistan.
3Department of Medical Education, University of Health Sciences, Lahore, Pakistan
4Avicenna International Medical University, Bishkek, Kyrgyz Republic
5Center for Refining and Advanced Chemicals, King Fahd University of Petroleum and Minerals, Dhahran, Saudi Arabia

Corresponding Author: zeeshan512@gmail.com
affect EPS synthesis, however, regular chemical ablation is required to remove it, which shortens membrane life and costs 50% extra [15, 16]. Polysulfone or cellulose base low-pressure membrane (LPM), encounters membrane fouling obstacles [17, 18]. Psf membranes have shown significant protein-fouling resistance, flux recovery, and excellent antibacterial activity. [19, 20, 21] Microbial mobs become entangled in small apertures, causing the membranes to become fragile and difficult to remove [7]. Following agglomeration through optimization of operational by-laws, chemical washing e.g. chlorine is a conventional backwash method to deal with membrane biofouling [22, 23, 24].

Another recent idea to reduce fouling is membrane affixation shift, which has limited techniques available, such as surface graft polymerization [25]. This shocking proof approach is widely used in an MBR [26]. However, this procedure necessitates elaborate energy systems, such as  $\gamma$-irradiation, UV-based, or plasma treatment, which increases fabrication costs [27, 28]. As a result, all of these techniques have drawbacks, and we should consider alternative solutions. Polysulfone has been evaluated as an excellent substitute in bioreactor membrane formation [29]. However, because polysulfone is hydrophobic, it is susceptible to hydrous media and various organic solvents [30]. The use of functional nanoparticles is growing, and bottom-up nanoparticle design is being used to create more intricate and versatile membranes [31], as well as adequate function performance. The entire treatment proficiency can be enhanced with a hybrid process that combines the unique features of NPs with other processes [32]. Microbially contaminated wastewater can be tempered instantly, economically, and conveniently by involving nanoparticles film or engraft in the membranes. Biofouling of membranes can be reduced by embedding nanoparticles, and polysulfone is chosen as a medium material due to its practical implications and wide range of applications in the construction of ultrafiltration membranes [33]. The silver existence and accessibility can be sensed by the degree of antimicrobial upshot that might serve as biofouling-resistant materials in nanocomposites [34, 35]. Nanotechnology is assisting in the growth of innovative tangibles and the use of nanoparticles, which are becoming increasingly important in water treatment [36]. In high-tech water treatment, high-grade effusions with membranes can be loaded with nanoparticles [37]. Nanoparticles are being used to create high-performance hybrid membranes with enhanced permeability, divisibility, and antibiofouling properties [38]. Exclusive functionalities of nanoparticles and polysulfone are combined to blend hybrid membranes that are much more efficient as compared to their traditional counterparts. Conventional membranes have static properties that are intended to provide separation whereas hybrid membranes provide decreased impressions leading to the amalgamation of several membranes in a single membrane [31, 39].

2. Materials and Methods:

2.1 Chemicals:

Growth Media and other chemicals were acquired from E. Merck (Darmstadt, Germany), Oxoid Lab Chemicals (Hampshire, U.K), BDH Lab Chemical Division (Poole Dorset, England), Difco Lab (Detroit Michigan, USA) and Sigma-Aldrich Chemicals Company (St. Louis, MO, USA).

2.2 Silver-inseminated Polysulfone membrane casting (Ag-Psf)

Implying a “wet phase-inversion” protocol, a Polysulfone ultrafiltration membrane was fabricated. N-Methyl-2-pyrrolidone (99%) was taken in a 100 mL glass pot including a magnetic stirrer and the temperature was set at 50°C. 10 g of polyvinyl pyrrolidone (PVP) was gradually mixed and the system continued to agitate. [40] The suspension was stirred for about an hour, after the addition of PVP. Mycologically synthesized AgNPs of 1-90 nm size were supplemented and mixed involving a sonication probe (Fisher Scientific Sonic Dismembrator Model 100). About 15 g of Psf grains were gradually infused with the solution and stirred for about 5 hours at 120°C. After the entire dissolution of Psf in the PVP/NMP solution, the container was detached from the hot plate and collected in a desiccator. The casting of the mixture dope was carried out at ambient atmosphere.
(humidity as 83% and temperature as 31°C) through a casting knife with a 110ìm gap set. The final step was drying after placing it at 28°C for one day in the water. The temperature and humidity were continuously monitored. The membrane mass was composed of overall 0.9% AgNPs while other compositions were 75% NMP, 15% Psf, and 10% PVP respectively.

2.3 Membrane Characterization:
For characterization, the specimen was placed on a copper stub after being sliced into small pieces to minimize the distortion of the microscopic examination. Before placing on a Scanning Electron Microscope (Jeol JSM 5910) the sample went through “gold sputtering” after acquiring a transverse section of the membrane by “liquid nitrogen freeze fracturing” (Bio-Rad Polaron Division). The SEM voltage was kept at 10-20 kV while the magnification was 500-7000x. The use of a scanning electron microscope (SEM) was for the establishment of the thickness of the membrane, porous substructure and some morphological attributes.

2.4 Fourier Transform Infra-Red (FTIR) Spectrometry:
For analysis, via FTIR the desiccated membranes were obtained at intervals of 0, 30, and 45 days from the L.B flask. These samples were further processed by placing them in front of a beam of FTIR to record spectra. The FTIR is specific for the analysis of changes produced due to AgNPs, biofouling, or degradation. These changes might be chemical or structural or in the membrane molecular skeleton.

2.5 Detection OfThe Wastewater Bacteria:
The identification of bacteria was performed by observing characteristics of colony staining by using gram stain and finally streaking on MacConkey agar for confirmation of gram-positive and negative. There were also some specific media like Eosin methylene blue (EMB) agar, Pseudomonas Cetrimide agar (PCA) and SS agar (Salmonella Shigella).

2.6 Antibacterial Efficacy:
To investigate the antibacterial behavior as well as the effect on growth curves of AgNPs (being used for modification of membrane), antibiotic sensitivity was checked first. Ampicillin (AMP), Gentamicin (CN), Ciprofloxacin (CIP), Nalidixic Acid (NA), and Meropenem (MEM) were selected to check the sensitivity of different isolates. Kirby Bauer disc diffusion method was employed.

2.7 Kirby Bauer’s disc Diffusion Method:
Muller Hinton agar plates were prepared and a bacterial lawn was streaked on it. To examine the antibacterial effect of B AgNPs it was incorporated into the preformed disc of Whatman filter paper No.1 by punching with the size of 6mm. The AgNPs were used in the form of suspension by adding distilled water and sonicated in the presence of ice for 3 minutes. The 6 discs containing B AgNPs as 30 microliters of 100 parts per million were placed in plates at a suitable distance. The temperature was maintained at 37ºC and the zone of inhibition was measured after 24 hours. This method was in accordance with the “Kirby Bauer disc diffusion” assay.

2.8 Study of Membrane Biofouling:
To assess biofouling, the membrane pieces were UV sterilized, and a 1 cm² piece of the membrane was incubated in two flasks under both shaking and static settings after being added to the LB medium. After 7 days, 10 mL of distilled water was taken and membrane pieces were shifted to that. Sonication of membranes was performed at 80W power for 3 minutes (Fisher Scientific Sonic Dismembrator Model 100). The serial dilution of samples was performed and 100 µL dilution was taken to be spread on plate count agar medium to measure CFU. The colonies were counted after being kept for 24 hours at 37ºC. For the ease of SEM examination, the same conditions were maintained for membrane pieces of the same type.

2.9 Biofilm Formation Under Shaking Conditions:
100 mL of LB broth medium was prepared in two flasks of 500 mL. Incubation of membrane pieces of 1cm² plain Psf and Ag-Psf membrane was followed. Shaking conditions were maintained for incubation and after every three to four days, the medium was refreshed.

3. Results:
3.1 Bacteriological Characterization of Waste Water
Bacterial cultures grown on nutrient agar were
isolated as pure cultures by sub-culturing again and again till the isolation of the pure colony was obtained (Table 1). Several isolates were identified; the detected bacteria were predominantly in the size range of 1ìm to 5ìm and observed as rods. They also secreted exopolysaccharides (EPS) and presented as biofilms. The species are *Enterobacter aerogenes* (*E. aerogenes*), *Escherichia coli* (*E. coli*), *Klebsiella pneumonia* (*K. pneumonia*), *Salmonella choleraesuis* (*S. choleraesuis*), *Shigella sonnei* (*S. sonnei*), *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Staphylococcus aureus* (*S. Aureus*).

**Table 1:** Colony characteristics of waste water bacterial isolates on selective culture media

<table>
<thead>
<tr>
<th>No.</th>
<th>Gram Stain</th>
<th>MacConkey agar</th>
<th>SS agar</th>
<th>EMB agar</th>
<th>PCA</th>
<th>S. aureus agar</th>
<th>Bacterial species</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>-ve</td>
<td>Dull pink</td>
<td>N.G</td>
<td>Dark center</td>
<td>N.G</td>
<td>N.G</td>
<td>Enterobacter aerogenes</td>
</tr>
<tr>
<td>2.</td>
<td>-ve</td>
<td>Red</td>
<td>N.G</td>
<td>Green metallic sheen</td>
<td>N.G</td>
<td>N.G</td>
<td>Escherichia coli</td>
</tr>
<tr>
<td>3.</td>
<td>-ve</td>
<td>Pink</td>
<td>N.G</td>
<td>Dark center</td>
<td>N.G</td>
<td>N.G</td>
<td>Klebsiella pneumonia</td>
</tr>
<tr>
<td>4.</td>
<td>-ve</td>
<td>Colorless</td>
<td>Teal blue with black center</td>
<td>Pink</td>
<td>N.G</td>
<td>N.G</td>
<td>Salmonella choleraesuis</td>
</tr>
<tr>
<td>5.</td>
<td>-ve</td>
<td>Light pink</td>
<td>Teal blue</td>
<td>Pink</td>
<td>N.G</td>
<td>N.G</td>
<td>Shigella sonnei</td>
</tr>
<tr>
<td>6.</td>
<td>-ve</td>
<td>Colorless</td>
<td>N.G</td>
<td>Pink mucoid</td>
<td>Mild Green fluorescence</td>
<td>N.G</td>
<td>Pseudomonas aeruginosa</td>
</tr>
<tr>
<td>7.</td>
<td>+ve</td>
<td>N.G</td>
<td>N.G</td>
<td>N.G</td>
<td>N.G</td>
<td>Manve</td>
<td>Staphylococcus aureus</td>
</tr>
</tbody>
</table>

### 3.2 Antibacterial Susceptibility Of Wastewater Bacterial Isolates:

The isolated colonies were checked for their resistance or sensitivity against different antibiotics. It was found that all the bacterial isolates were sensitive to ciprofloxacin in the range of 16 to 18 mm. *E. Aerogenes* exhibited maximum sensitivity of 18.2 mm, 17 mm and 16 mm against ciprofloxacin, meropenem and gentamicin as compared to other isolates. *E. coli, K. pneumoniae* and *S. choleraesuis* were sensitive against ciprofloxacin and at a minimum level towards ampicillin, gentamicin, nalidixic acid and meropenem. *S. sonnei* was sensitive towards ciprofloxacin and ampicillin, mildly sensitive against gentamicin while resistant towards meropenem and nalidixic acid. *P. aeruginosa* was sensitive towards ciprofloxacin while moderately sensitive towards other antibiotics. Added sensitivity pattern of *S. aureus* was observed against ciprofloxacin, gentamicin and nalidixic acid, its sensitivity was moderate against meropenem while resistance was exhibited against ampicillin (Figure 1).

![Antibacterial susceptibility profile of wastewater isolates for different antibiotics](image)

**Figure 1:** Antibacterial susceptibility profile of wastewater isolates for different antibiotics
3.3 Antibacterial Activity of B AgNPs:
In the case of B AgNPs, a concentration of 50 ppm demonstrated activity. Isolates were responsive to a 500 ppm concentration of B AgNPs, and the isolates examined displayed minor antibiotic resistance. All of the identified isolates were responsive to all of the concentrations used to test their sensitivity.

The overall pattern of sensitivity observed was that the zone of inhibition grew as the concentration of B AgNPs increased. Maximum sensitivity was observed for *E. aerogenes*, *E. coli*, *S. choleraesuis*, *S. sonnei* and *P. aeruginosa* (around 30 mm) while *K. pneumonia* and *S. aureus* showed minimum sensitivity (around 24 mm) at 500 ppm (Figure 2).

![Figure 2: Antibacterial activity of B AgNPs against selected wastewater isolates](image)

3.4 Polysulfone (Psf) Membrane Characterization:
The Psf membranes were characterized using SEM. The membranes containing AgNPs were found to be 110 µm thick. SEM revealed that AgNPs were found in the membrane voids. The pores in the membrane were visible in cross-sectional images of the membrane, indicating that AgNPs do not affect the development and structure of pores. The majority of spherically shaped pores were determined to be between 0.5 and 5 µm in size, with only a few exceeding 10 µm. (Figure 3).

![Figure 3: SEM images of plain Psf membrane surfaces at different resolutions (a-c) cross-sectional view (d)](image)
3.5 FTIR of Psf membranes:
FTIR performed for synthesized Psf membrane showed results as peaks related to varying groups. These groups are major parts of Psf membrane and also correlated with Sigma-Aldrich standard spectrographs i.e., the peak of CH, and sulfone groups were observed at 1488cm⁻¹ and 1150cm⁻¹. At 1241cm⁻¹ observed peak was for the benzoate group (ester bond). Although some peaks were different from that of standard spectroscopy (available from Sigma-Aldrich), these differences were correlated with constituents used for the preparation of the membrane (Figure 4).

3.6 Formation of Biofilms on Psf Membranes:
The formation of biofilms was analyzed by counting through CFU, spectrographs via FTIR and SEM observation. These observations were carried out while monitoring the Psf membrane at 37°C and observations were taken at intervals of 7, 21, 30, and 45 days. The results are given below in Figure 5.

3.7 Biofilm's Bacterial Count (CFU):
CFU (Colony Forming Units) were isolated from plain membranes and Psf membranes with BAgNPs that had been treated separately. This corresponds to the CFU number of the membrane's area as 1 cm². To calculate the CFU of 1 cm², two membranes were processed separately, one with AgNPs added and the other without them. The CFU differed significantly between the two membranes: plain Psf membrane and Ag integrated Psf membrane. While in comparison to Psf-membrane, the growth was not significant with the Ag-Psf membrane on the 7th and 21st day. The plain Psf-membrane CFU of 118 and 308 cfu/cm² on 30th and 45th day while that of the Ag-Psf membrane was 20 and 36 cfu/cm² (Figure 5).
3.8 Polysulfone (Psf) Membrane FTIR Analysis During Biofilm Formation

To analyze plain Psf membrane via FTIR, samples were taken at different intervals (after incubation of 7, 21, 30 and 45 days). The peaks observed in the FTIR spectra become more prominent proportionately with increasing incubation time (Figures 6 and 7). The similarity was observed in the results of Psf FTIR and the number of peaks of the sample membrane (Figures 4, 6 and 7). The transmittance of roughly 660 cm$^{-1}$ in the region of the fingerprint was noticed as a symptom of the breakdown of the molecular structure, which occurs due to CH out of plane displaying a -CH=CH- in cis form. The continual increase of 3000 to 3500 cm$^{-1}$ can be attributed to an increase in the number of phenols and alcohols in this area (Figures 6 & 7).

Figure 6: FTIR spectrum of Psf membrane on the 7th and 21st day during biofilm development.

Figure 7: FTIR spectrum of Psf membrane on the 30th and 45th day during biofilm development.
3.9 Plain Psf Membranes Under Sem During Biofilm Formation:
Development of EPS and bacterial growth was observed in the SEM of the plain Psf membrane. Different resolutions were used to observe biofilm development (Figure 8: a to d). Rupture of membranes is found to be the initial step of the formation of biofilms in bacteria (Figure 8: e & f).

![Figure 8: Biofilm succession and Psf membrane at different resolutions (a-d), marked cracks in Psf membrane due to biofilm development (e & f).](image)

3.10 Ag-Psf Membranes During Biofilms Formation Using FTIR:
Samples were obtained at various intervals after incubation of 7, 21, 30, and 45 days to examine the Ag-Psf membrane through FTIR. With increasing incubation time, the peaks identified in the FTIR spectra grow increasingly evident (Figures 9 & 10). A symptom of molecular structural breakdown was noticed as the transmittance of roughly 660 cm⁻¹ in the fingerprint area, which is a very high range and happens to owe to CH out of plane portraying a -CH=CH- in cis form. A constant rise of 3000 to 3500 cm⁻¹ can be attributed to an increase in the number of phenols and alcohols in this area (Figures 9 & 10).
3.11 Ag-Psf Membranes During Biofilms Formation Under SEM:
At intervals of 7, 21, 30 and 45 days, the spectra of SEM showed all the changes undergone the formation of biofilms and their continuous development. As a whole, the Ag-Psf membrane showed very less development of biofilms (Figure 12) in comparison to the plain Psf membrane (Figure 8). There was a very dense EPS secreted and entered the spaces in the pores of the membrane. This biofilm was thin in the case of the Ag-Psf membrane and correlated with the growth of bacteria and secretion of EPS. The images from SEM showed that the growth was less in AgNP's incorporated areas. Bacteria that have been destroyed are highlighted. According to observation, the areas undergoing reaction showed a cloudy appearance due to the NPs present there (Figure 11). By observing from varying resolutions the less development of biofilm of the Ag-Psf membrane was confirmed (Figure 12).

Figure 9: FTIR spectrum of Ag-Psf membrane on the 7th and 21st day during biofilm development

Figure 10: FTIR spectrum of Ag-Psf membrane on the 30th and 45th day during biofilm development.

Figure 11: Ag-Psf membrane interaction of AgNPs with bacteria at different resolutions (a-d)
4. Discussion:

As freshwater becomes more valuable by the day, the demand to clean grows accordingly. There is an urgent need for environmental stewards to create a framework for water body restoration [41]. The previously proposed treatment methods are less successful due to high prices and methodological problems. The most significant impediment to the operation of membrane bioreactors, desalination, and industries is "biofouling of membranes" [17, 22, 24], which also covers the use of membranes for refining applications like beverages and milk processing [42, 43].

Polysulfone membrane is the preferred membrane for usage in most wastewater treatment facilities and businesses at various levels of treatment due to its capacity to withstand high flow flux, heat resistance, gamma sterility, and low molecular binding [18, 44]. The growth of biofilms on this polymer by wastewater bacteria reduces the average lifespan of the membrane. In membrane reactors, the only method to remove them is to clean or replace the membranes, which is an expensive procedure [45]. By addressing the biofouling issue, the membrane bioreactor may be optimized. Physical/chemical cleaning in place, membrane surface modification, influent preparation, and other approaches have been explored to eliminate biofouling [32, 46], and rather promising results can be achieved by the use of AgNPs [33, 47].

AgNPs behave differently than bulk materials due to quantum effects and changed chemistry. Their antibacterial character has also been recognized for centuries [34, 48]. AgNPs' chemical and biological characteristics, as well as their electrical, optical, and magnetic capabilities and reactivity, make them appealing for usage in the medical, food, textile, and consumer goods sectors [49]. AgNPs can also make polysulfone membrane immune against biofouling by incorporating these NPs in Psf membranes which is carried out in this research [50]. *Aspergillus niger* was used to synthesize AgNPs to be utilized in this study. The isolated strains of bacteria at the first stage of this research were seven in number and identified as Enterobacter *aerogenes*, *Escherichia coli*, Klebsiella *pneumonia*, *Shigella sonnei*, Salmonella *choleraesuis*, *Pseudomonas aeruginosa*, and Staphylococcus *aureus* (Table 1). All the isolated species of bacteria are the common habitat of water bodies. Horizontal gene transfer makes some of the isolated species to be resistant [51, 52].

The next stage was the fabrication of membranes, plain or incorporated with Ag-NPs, characterized through FTIR spectra (Figures 4, 6, 7, 9, and 10) and SEM images (Figures 3, 8, 11 & 12). As reported by Kim et al. (2007) the NPs of the size 50-500 nm get aligned on the surface of the membrane because the size of membrane pores is smaller than NPs; the same was reported in this research via SEM images [46]. According to studies, these NPs on the surface of the membrane work more efficiently to the hindrance of microbes damaging the membrane due to biofouling but it is not long-lasting as with time,
these NPs get washed off from membrane surfaces due to any attachment problem [53]. To function properly, the membrane pores should also remain intact and the requirement is also fulfilled by NPs as they are attached to the surface and do not enter or affect pores on the membrane [49, 54, 55]. The prepared Psf membrane FTIR (Figure 4) revealed good agreement towards the Sigma-Aldrich Psf membrane FTIR available on the website. The high concentration of benzoate was demonstrated by its maximum peak at 1241 cm\(^{-1}\). The high concentration of element was demonstrated by multiple repeating benzoate in the membrane structure, and the transmission permitted by it was limited up to 52%. The components contained in the polysulfone structure had the second largest peak at 1488 cm\(^{-1}\) and 1150 cm\(^{-1}\), respectively.

The difference in cfu count between Psf and Ag-Psf grew over time, with a 40 - 50% difference on 30 days and a 75 - 80% difference on 45 days. The count of Ag-Psf was not significant even after 45 days (Figure 5). Since silver has an antibacterial effect, Ag-Psf showed resistance to strains growing on it; nevertheless, in the case of the plain membrane, bacterial strains with high resistance were able to grow at first, but the membrane got degraded with time, allowing additional strains to grow.

The production of biofilms was observed by analyzing through SEM, while the chemical changes due to biofouling were investigated by FTIR. The visible changes in the membrane fabrication were observed through FTIR (Figure 4). In comparison to the plain membrane (Figures 6 and 7), the Ag-Psf membrane showed less distortion in membrane fabrication (Figures 9 and 10). FTIR graphs revealed biofouling peaks, which provide clear images of biofouling distortion in the membrane's molecular structure (Figures 7, 9, and 10). Peaks showed by sulfone, benzoate, C-C-C bending and ring puckering, and CH, were at 1240 cm\(^{-1}\), 1149 cm\(^{-1}\) 1105 cm\(^{-1}\) and 1487 cm\(^{-1}\) respectively (Figure 8) but in the case of the membrane with biofouling, high absorbance was observed with different peaks showing a breakdown of molecular structure that may result from CH out of plane depicting a \(\text{CH}=\text{CH}\) in the cis form at 687 cm\(^{-1}\). It was also observed via FTIR that the biofouling rate in the shaker incubator was reasonably fast, which explains why it gave the same findings under agitated circumstances, although they differed in the concentration of new molecule synthesis. The chemical alterations were the same after 14 days.

Bacteria were not able to form biofilms in the area with an accumulation of NPs (Figures 11 and 12), therefore the Psf-membrane without Ag-NPs facilitated the growth of rich biofilms over the Ag-Psf membrane. The bacterial count and biofilm formation on the Psf membrane was a continuous process. When the bacterial cell is close to NPs it is presented by a highlighted region (Figure 12), while the presence of NPs is seen as a cloudy region. A charge imbalance occurs during the interaction of bacteria with NPs due to a difference in the electrical charge that gives a cloudy appearance, seen through images of SEM. Bacterial growth was dense in Psf membranes without NPs but less dense with NPs. Resistance in the Ag-Psf membrane to the development of biofilms and subsequent biofouling was exhibited through SEM (Figures 11 & 12).

5. Conclusions:
Polysulfone (Psf) membrane infused with AgNPs helped in reducing biofilm generation, hence creating an anti-biofouling effect on it. Due to their applications in antibacterial activity, longer stability, extending the life of filtration membranes, and significant biofouling resistance, these Psf membranes blended with AgNPs may be recommended in wastewater treatment. Furthermore, this work may help in paving the way for better modifications in membranes used for wastewater treatment.

Acknowledgments
The authors are grateful to the Higher Education Commission (HEC), Government of Pakistan for giving financial support under HEC-5000 Indigenous scholarship scheme batch VI with Award Number; 106-1317-BM6-002.

Conflict of interest
There is no conflict of interest among the authors.

References:
1. M. Kummu et al., “The world’s road to water scarcity: shortage and stress in the 20th


31. V. V. Tarabara, “Multifunctional nanomaterial-enabled membranes for water treatment”. In Nanotechnology applications for clean water (pp. 59-75), 2009.


