Superhydrophobic nanocarbon-based membrane with antibacterial characteristics

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1 | INTRODUCTION

Hydrophobic membranes are the most ideal membranes for membrane distillation (MD) processes in avoiding membrane pore wetting.1,2 Embedding nanoparticles within the membrane polymeric structure are known to enhance the mechanical properties of membrane such as tensile strength and initial Young's modulus in addition to playing an effective role in increasing the hydrophobicity of the modified membrane.3,4 Additionally, the hydrophobic nature of the membrane surface enhances its operational conditions. The MD process offers several unique advantages compared to other membrane-based, pressure-driven processes suitable for wastewater, seawater, and many other water treatment applications.5

Notably, one of the major issues affecting the performance and efficiency of the MD process is the membrane surface fouling.6 Fouling behavior of MD is the deposition of contaminants (organic or...
inorganic materials) on the membrane surface thereby leading to a decrease in water flux through the membrane. This results in an increase in the operating cost through the addition of more treating requirements, such as periodical membrane cleaning or replacement.7,9 The occurrence of serious membrane fouling depends on the membrane material, its surface chemistry, and surface morphology.

Membrane biofouling is an extremely complex phenomenon.10 Biofouling is the result of the deposition of active organisms, such as bacteria, fungi, viruses, and other microorganisms, which have a normal affinity for adhering to the membrane surface.11 The bacterial growth results in a biofilm surface, which represents a major problem in the practical application of membrane processes.12 This phenomenon results in the decline of the vapor pressure driving force, considerable upsurge in energy consumption due to the increase in temperature polarization and negatively affects the membrane efficiency through pore blockage and wetting.13,14 Bacteria can grow and survive on the MD membrane despite the fluctuating inlet feed temperature of 70–90°C, which could drop to 30–50°C at the outlet of the model. Moreover, it can survive in harsh conditions and high salt concentrations such as seawater. Thus, the exposed surface of the membrane, which may encounter different thermal conditions, is suitable for growth of microorganisms, at temperatures lower than 60°C. High temperatures are not applicable as they reduce the effectiveness of microorganisms. In addition, biofouling can take place irregularly, even with very low levels of nutrients.15

A common approach to prevent membrane biofouling is by applying a preventive primary treatment to the feed water.16 This pretreatment method helps reducing the number of bacteria colonies; however, it does not affect the nutrients exists in water that feed the bacteria.17 Moreover, this method is laborious and costly. In addition, even with 99.99% removal of bacteria, the pretreatment step does not inhibit bacterial growth on the membrane surface due to its ability to grow, multiply, and relocate rapidly. This type of bacterial growth usually takes place at relatively high feed temperature due to the presence of nutrients in wastewater or seawater.2,18 The surface morphology of the membrane surface has a pronounced impact on the microorganisms’ growth. This is due to the fact that bacteria are more likely to be trapped on rough surfaces when compared to smooth ones.19,20 A few reports have described nanoparticles in membrane technologies.21,22 Incorporating nanoparticles, such as carbon nanomaterials (CNMs), has proven to be a promising approach in deactivating bacteria upon direct contact with bacterial cells. However, this approach does not completely remove all bacterial cells.23,24

Previous studies on different factors affecting membrane fabrication were preliminarily, and restricted to single factors.25,26 Univariate process optimization methods are incapable of explaining the possible interaction effects among fabrication parameters.27 The design of experiment (DoE) statistical technique is a preferred approach in determining the influence of fabrication variables and developing a final mathematical model for the fabrication process. Previously, many studies reported that CNMs were effective in enhancing membrane antimicrobial activities. The aim of the present study was to impart antibacterial properties to PVDF-HFP, mixed with superhydrophobic carbon nanomaterials/powder activated carbon (CNMs/PAC) materials to be employed as a new composite membrane for MD applications. To fulfill this aim, a central composite design (CCD) response surface methodology (RSM) was utilized to analyze the influences of the main membrane fabrication factors and their interaction effects on the bacterial growth at different levels of CNMs. The effect of three parameters, that is, CNMs loading, polymer concentration, and the thickness of the casting knife on biofouling was studied. By conducting an analysis of variance (ANOVA), and by optimizing the fabrication parameters, this study concluded an optimum anti-biofouling membrane design within the studied experimental conditions.

2 | MATERIALS AND METHODS

2.1 | Materials

Carbon nanomaterials CNMs/PAC used was a combination of several carbon nanomaterials such as carbon nanotube (CNTs), carbon nanofibers (CNF), and carbon spheres (CNS) formed as a result of CVD reaction that took place over powder activated carbon (PAC). The preparation process and the characterizations of CNMs/PAC were thoroughly discussed in our previous article.28 Poly-vinylidene fluoride-co-hexafluoropropylene (PVDF-HFP) polymer was used as a matrix for CNM-modified composite polymer. The solvent used for preparation of membrane was N-methyl-2-pyrrolidinone (NMP, anhydrous, 99.5% and density of 1.03 g/mL). Both polymer and solvent were purchased from Sigma-Aldrich. Staphylococcus aureus (S. aureus, NCTC 12973) culture was prepared in homogeneous nutrient broth media for the evaluation of the bacterial adhesion and for the characterization of the composite membranes. Nutrient broth and nutrient agar media were purchased from Sigma-Aldrich. Composite membranes were placed on the solidified agar media in petri dishes followed by the inoculation of S. aureus NCTC 12973 while nutrient broth media in flask was inoculated simultaneously by both composite membrane and S. aureus NCTC 12973. Inoculated nutrient broth flask and nutrient agar plates were incubated at 37°C for 5 days. Composite membranes in nutrient agar plate were therefore characterized by SEM while in nutrient broth media, the bacterial colonies were evaluated by UV–vis spectrophotometric optical density (OD600). All the experiments were repeated in triplicates.

2.2 | Statistical analysis and optimization of fabrication composite membrane

Design Expert software (DoE: Version 7) was used to design and plan the experimental methodology and to minimize the number of experiments. Consequently, CCD was conducted using the three preparation parameters namely, CNMs/PAC loading, concentration of PVDF-HFP and thickness of casting knife. The RSM statistical method was utilized for modeling, studying the interactions and to optimize the
membrane fabrication parameters that affect the growth of *S. aureus* survival as a response. The optimization criteria involved minimizing *S. aureus* survival as a response within the chosen range of membrane fabrication parameter. Moreover, ANOVA for the experimental results was performed to determine the significance of the model and process interactions of the various fabrication parameters. The coefficients of determination ($R^2$) which can be defined given in Equation 1 was one of most important indications for validation.

$$R^2 = \frac{\sum_{i=1}^{n} (y_i - \bar{y})^2 - \sum_{i=1}^{n} (y_i - \bar{y})^2}{\sum_{i=1}^{n} (y_i - \bar{y})^2}$$

where $y_i$, $y'_i$, and $\bar{y}$ are the $i$th experimental value, $i$th predicted value, and the data average value, respectively.

### 2.3 Preparation of the of the composite membrane

The flat sheet composite membranes are prepared using phase inversion process. Upon following the DoE procedure, which suggested different level of CNMs/PAC, concentration of polymer and membrane casting knife. The CNMs were dispersed in NMP solvent for 1 hr with an ultrasonic processor (UP400S, 24 kHz) until the best distribution is achieved; then, granular PVDF-HFP polymer added to the homogenous NMP-CNMs/PAC suspension using a magnetic stirrer at lab conditions, the DoE details is presented in Table 1. The mixture was left over night at lab conditions to make sure all the air bubbles was left before casting it by casting knife on a glass plate on the casting machine. The coagulation process started by putting glass plate with composite mixture in deionized water. Later, the precipitation process of the membrane samples in a deionized water bath to complete their formation and confirm that all residual solvent traces were removed from the membrane. Finally, the prepared membranes were dried in lab conditions for 2 days.

### 2.4 Initial adhesion and surface hydrophobicity assay experiments

The biofouling activity of the composite membranes against *S. aureus* was tested for viable-cell counting as described below. The anti-biofouling activity against *S. aureus* was also determined for the composite and pristine membranes. Upon appropriate dilution of *S. aureus*, which was cultured in the nutrient broth, each sample was shaken at 250 rpm and then immersed in the *S. aureus* suspension at 37°C overnight. This was followed by harvesting through centrifugation at 3,500 rpm at room temperature for 30 min. A culture of *S. aureus* of about $1.0 \times 10^8$ cells/ml was prepared in the nutrient for antibacterial testing.

The growth of bacteria was assessed under the optical density of the suspension based on a standard calibration from spread plate counting. The 2 cm in diameter samples were prepared and exposed in the *S. aureus* cell suspension (100 ml containing about 8.68664 CFU). The pathogenic bacteria growth was estimated during a period of 120 hr. The suspensions were diluted by taking the colonies from the bacteria stock and placing it into a flask containing 2 cm diameter of composite membrane. All flasks were incubated for 24 hr at 40°C. The surviving *S. aureus* were calculated by counting the number of colonies forming units CFU in five replicates for each drop on ELISA (xMark, Biorad). The CFU values were then converted into log values.

The 2 cm composite membrane was then placed on the agar petri dishes. The bacterial suspension (8.68664 CFU) was spread on the membrane surface and cultured overnight. All prepared membranes were tested in triplicate. All petri dish samples were incubated for over 72 hr at 37°C. The distribution and adhesion pattern of the *S. aureus* was evaluated by the SEM Jeol JSM-5510 (Jeol Ltd., Japan). The composite membranes were then incubated in 5.0 ml *S. aureus*

### Table 1

<table>
<thead>
<tr>
<th>Samples</th>
<th>$\times100$ ratio of CNMs/PAC loading (mg/g)</th>
<th>Polymer to solvent ratio (%)</th>
<th>Casting knife thickness (μm)</th>
<th>Response number of survived cells ($\times10^8$ CFU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>100.00</td>
<td>24.00</td>
<td>100.00</td>
<td>1.55</td>
</tr>
<tr>
<td>A2</td>
<td>100.00</td>
<td>20.00</td>
<td>300.00</td>
<td>0.429</td>
</tr>
<tr>
<td>A3</td>
<td>1,000.00</td>
<td>20.00</td>
<td>300.00</td>
<td>0.459</td>
</tr>
<tr>
<td>A4</td>
<td>1,000.00</td>
<td>24.00</td>
<td>100.00</td>
<td>0.37</td>
</tr>
<tr>
<td>A5</td>
<td>1,000.00</td>
<td>20.00</td>
<td>100.00</td>
<td>0.112</td>
</tr>
<tr>
<td>A6</td>
<td>100.00</td>
<td>24.00</td>
<td>300.00</td>
<td>0.203</td>
</tr>
<tr>
<td>A7</td>
<td>100.00</td>
<td>20.00</td>
<td>100.00</td>
<td>1.8</td>
</tr>
<tr>
<td>A8</td>
<td>1,000.00</td>
<td>22.00</td>
<td>200.00</td>
<td>2.65</td>
</tr>
<tr>
<td>A9</td>
<td>1,000.00</td>
<td>24.00</td>
<td>300.00</td>
<td>1.29</td>
</tr>
<tr>
<td>A10</td>
<td>550.00</td>
<td>22.00</td>
<td>200.00</td>
<td>3.29</td>
</tr>
<tr>
<td>A11</td>
<td>550.00</td>
<td>22.00</td>
<td>300.00</td>
<td>2.76</td>
</tr>
<tr>
<td>PVDF-HFP</td>
<td>-</td>
<td>22.00</td>
<td>250</td>
<td>1.44</td>
</tr>
</tbody>
</table>
culture for 72 hr. Subsequently, all samples were kept hot and were dried at 55°C for 48 hr. The samples were then coated with platinum with the sputter coater chamber, and the bacteria cell size and morphology were observed using the SEM. The microbial cell activity of the CNMs/PAC was monitored against the growth of the S. aureus. All the experiment related to microbial growth were repeated three time.

3 | RESULTS AND DISCUSSION

Microorganisms growth on membrane during water separation processes not only results in eliminating membrane life but it decreases permeate water flux and leads to higher energy consumption as well. Consequently, the membrane needs to be modified to improve its antibacterial efficiency.

Ten milligrams of superhydrophobic CNMs/PAC were added to the S. aureus cell suspension of 100 ml, containing about 8.68664 CFU. The pathogenic bacteria growth was estimated during 120 hr. It represents the bacteria with nutrients and the observation in the text was supported by similar other studies as shown in Figure 1. The bacterial growth under the effects of the superhydrophobic CNMs/PAC, prior to membrane fabrication, was obtained by performing cytotoxicity test individually. This test was intended to verify the independent impact of CNMs/PAC on the microorganisms and to examine the antibacterial activity of this material. From these results, it is obvious that the presence of CNMs/PAC has remarkably decreased the number of the S. aureus cell colony from 8.68664 to 7.99913 after 24 hr and 6.69197 after 120 hr. This bacterial growth inhibitory behavior provides great evidence on the antibacterial activity of CNMs/PAC.

After fabricating the membrane, the effects on the growth of the S. aureus on the membrane surface including CNMs/PAC loading, polymer concentration, and the thickness obtained by the casting knife gap were studied using an RSM method. Following CCD, DoE suggested number of runs and parameters were set earlier. A total of 11 runs were performed to study the impact of the membrane surface on the microbial cell activity. The fabrication conditions of composite membrane (11 samples) and the results are shown in Table 1.

The ANOVA analysis examined and compared the response data to various polynomial models (mean, linear, two factor-interaction [2-FI], quadratic, and cubic) as shown in Table 2. The F value in one-way ANOVA is a test for evaluating the significant difference of the variance between the means of two populations. In addition, it determines the p value, which is the probability of getting a result at least as extreme as the one that was observed, given that the null hypothesis is true. The ANOVA results reveals that the linear, 2FI, and cubic models were not significant as the p-value (prob >F) calculated were more than .05.

The regressed S. aureus growth model might not be competitive as based on the coefficients of determination ($R^2$) given in Equation 1, which indicates that the linear model had an unacceptable $R^2$ value of .3553. Furthermore, the cubic model was aliased because the full-factorial design matrix delivered little exceptional design arguments to adequately determine the conclusions of the terms in the cubic model.

The quadratic model was selected as the best fitting model based on the “Prob >F” value of 0.0570 before modification, and 0.0022 after modification. The value of $R^2$ for the experimental and predicted values of the number of survived cells is .9995 according to the Equation 1, which indicates 99.95% of the original uncertainty has been explained by the model predictions. Moreover, the Figure 2 proves a moral convergence between the experimental and predicted values of the number of survived cells.

**TABLE 2** Analysis of variance for partial sum of squares

<table>
<thead>
<tr>
<th>Source</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>p-value prob &gt;f</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quadratic</td>
<td>1.57</td>
<td>3</td>
<td>0.52</td>
<td>165.85</td>
<td>.0570</td>
<td>.9955</td>
</tr>
<tr>
<td>Modified quadratic</td>
<td>6.46</td>
<td>8</td>
<td>0.81</td>
<td>464.36</td>
<td>.0022</td>
<td>.9995</td>
</tr>
<tr>
<td>Cubic</td>
<td>$3.153 \times 10^3$</td>
<td>1</td>
<td>$3.153 \times 10^{-3}$</td>
<td>0.43</td>
<td>.7387</td>
<td>–</td>
</tr>
<tr>
<td>Linear</td>
<td>1.00</td>
<td>3</td>
<td>0.63</td>
<td>1.43</td>
<td>.7387</td>
<td>.3553</td>
</tr>
<tr>
<td>2FI</td>
<td>3.89</td>
<td>3</td>
<td>1.30</td>
<td>3.30</td>
<td>.1396</td>
<td>.7568</td>
</tr>
</tbody>
</table>

Abbreviations: df, degree of freedom of different source; F, F-value; MS, mean of square; p, probability; SS, sum of squares.
Fabrication parameters, such as CNMs/PAC loading, polymer concentration, and casting were optimized using the method of multiple variables to study the effect on the survival of cells. The analyses of variance (ANOVA) of the number of the survived S. aureus cells are summarized in Table 3. The high modified quadratic model $F$-value of 464.36 implies the model is significant. There is only a 0.22% probability that the model $F$-value could occur due to noise. In this case, the main effects of CNMs/PAC loading (A), polymer concentration (B), the effect of second-order response of CNMs/PAC loading ($A^2$), the effect of second-order response of polymer concentration ($B^2$), the effect of the interaction between CNMs/PAC loading with polymer concentration (AB), and the polymer concentration with the thickness of the casting knife (BC) were significant and represent the major determinants of the cells survival response. On the other hand, $p$-values greater than .05 indicated that model terms that were not significant. This includes the main effect of thickness of casting knife (C), the second-order effect of thickness of casting knife ($C^2$) and the interaction effect of CNMs/PAC loading with thickness of casting knife (AC).

Consequently, it could be concluded that thickness of casting knife (C and $C^2$) and the interaction of CNMs/PAC with thickness of casting knife (AC) had less effect on of the number of the survived cells responses over the studied range. It could be concluded that the thickness of the casting knife (C and $C^2$) and the interaction of CNMs/PAC with the thickness of the casting knife (AC) had less effect on of the number of survived cells. Nevertheless, these model terms were not eliminated from the analysis to ensure that the selected quadratic model remained hierarchical.

Where the $F$ is the test for comparing model variance with residual (error) variance, $df$ is the degrees of freedom, mean square is estimate of the block variance, $F$ value is the term for comparing the variance and prob $>F$ is probability of seeing the observed $F$ value.

The regression model tested statistically (transformed to invers sqrt) for the number of survived S. aureus and is expressed by the Equation 2:

$$S.aureus \text{survival} = 0.56 + 0.25A - 0.18B + 0.026C - 0.30AB - 0.59AC + 0.21BC + 0.70A^2 + 0.23B^2 - 1 \tag{2}$$

where A represents CNMs/PAC loading, B concentration of PVDF-HFP, and C is the thickness of the casting knife.

The effects of the process parameters on the survival of S. aureus cells were scrutinized to determine the desired conditions that favored the decrease in the number of the survived S. aureus cells. Biofouling occurs when the microorganisms exist as communities in the form of bio-films on the hydrophobic surface. Accumulation of these microorganisms results in a semi-solid layer between the liquid medium and the hydrophobic surface, which prevents the vapor passing through the membrane. The results showed a remarkable agreement between the predicted and experimental values of the S. aureus survival with a mean error of 0.22%.

One way for identifying outliers in the original data is to use the studentized residuals (SR). The SR is calculated by deleting the one experimental observations at a time, and refitting the model using the remaining data points. Then, the observed response values are compared to their fitted values based on the models with the ith

![FIGURE 2](image1.png)

**FIGURE 2** Parity plot of actual and experimental values of number of the survived *Staphylococcus aureus* survival

**TABLE 3** The analysis of variance for the response surface quadratic model

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum of squares</th>
<th>$df$</th>
<th>Mean square</th>
<th>$F$ value</th>
<th>$p$-value</th>
<th>prob $&gt;F$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>6.46</td>
<td>8</td>
<td>0.81</td>
<td>464.36</td>
<td>.0022</td>
<td></td>
</tr>
<tr>
<td>A-CNMs/PAC</td>
<td>0.48</td>
<td>1</td>
<td>0.48</td>
<td>277.85</td>
<td>.0036</td>
<td></td>
</tr>
<tr>
<td>B-PVDF-HFP</td>
<td>0.27</td>
<td>1</td>
<td>0.27</td>
<td>153.52</td>
<td>.0065</td>
<td></td>
</tr>
<tr>
<td>C-casting knife</td>
<td>$5.687 \times 10^{-3}$</td>
<td>1</td>
<td>$5.687 \times 10^{-3}$</td>
<td>3.27</td>
<td>.2123</td>
<td></td>
</tr>
<tr>
<td>AB</td>
<td>0.73</td>
<td>1</td>
<td>0.73</td>
<td>420.13</td>
<td>.0024</td>
<td></td>
</tr>
<tr>
<td>AC</td>
<td>2.82</td>
<td>1</td>
<td>2.82</td>
<td>1,618.67</td>
<td>.0006</td>
<td></td>
</tr>
<tr>
<td>BC</td>
<td>0.34</td>
<td>1</td>
<td>0.34</td>
<td>197.06</td>
<td>.0050</td>
<td></td>
</tr>
<tr>
<td>$A^2$</td>
<td>0.40</td>
<td>1</td>
<td>0.40</td>
<td>228.21</td>
<td>.0044</td>
<td></td>
</tr>
<tr>
<td>$B^2$</td>
<td>0.033</td>
<td>1</td>
<td>0.033</td>
<td>18.92</td>
<td>.0490</td>
<td></td>
</tr>
<tr>
<td>Residual</td>
<td>$3.478 \times 10^{-3}$</td>
<td>2</td>
<td>$1.739 \times 10^{-3}$</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>6.46</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
observation deleted. The deleted residuals are then calculated based on comparing the observed response values to their regressed ones. Finally, the SR is calculated by standardizing the deleted residuals. In general, and observation is regarded as an outlier if the SR is larger than 3.5. Based on Figure 3, all SR values are below 3.5, indicating that none of the observed data points is an outlier.

The most effective characteristic in membrane include surface behavior (chemical and physical properties), the porosity, and the mechanical and thermal properties. Based on that, CNMs/PAC loading is expected to have significant impact on both surface behavior and mechanical properties. Furthermore, the most effective parameter in forming the pores structure is the polymer-solvent ratio. Finally, the thickness plays a major role in determining the mechanical and thermal properties.

3.1 Interactive effects of CNMs/PAC loading, concentration of PVDF-HFP and the thickness of the casting knife setup on the survival of S. aureus

The interactive effects of the fabrication parameters on the S. aureus survival are graphically illustrated by 3D response surface curves. Figure 4 shows that CCD curves were plotted on the foundation of the essential features of the empirical model. Figure 4a shows the special effects of CNMs/PAC loading and polymer concentration on the S. aureus survival. There was a notable decrease in the bacterial survival with increasing CNMs/PAC from 100 to 1,000 mg. This suggests that conducting the fabrication with low CNMs/PAC loading was unsuitable. This finding agrees with previous reports on CNMs/PAC exhibiting strong antimicrobial activity.33,34 The effect of CNMs/PAC and the thickness of the casting knife on the survival of S. aureus is

![Figure 3](image1.png)  
**FIGURE 3**  Staudentized residuals for the suggested Staphylococcus aureus survival model

![Figure 4](image2.png)  
**FIGURE 4**  Response surface plots for the effects of fabrication parameters on the survival of Staphylococcus aureus
shown in Figure 4b. The microbial cells have decreased steadily with the thickness of the casting knife for the composite membrane matrix. This is because the increase in membrane thickness increased the amount of CNMs/PAC loading throughout the membrane matrix, and thus decreased the survival of the *S. aureus*. However, a high thickness of membrane in MD was unfavorable. The concentration of PVDF-HFP is more effective than the increasing of the thickness on the number of survived bacteria. The lowest number of microbial cells is obtained by decreasing the polymer concentration, while adding maximum amounts of CNMs/PAC as shown in Figure 4c.
3.2 | Membrane anti-biofouling study

The antibacterial activity of the prepared composite membrane (A1–A11) is measured by the performance of microbial assessments against the *S. aureus*. The results were obtained by determining the zones of inhibition of the composite membrane with the *S. aureus* cells after 72 hr of contact. In case of contact results, overall A11 showed significant deviation of bacterial cell growth and colony pattern as compared to other treatments. This was followed by A8 and A9 composite membrane.

The microbial cells were connected to each other, whilst forming a close interactive environment during the association with the pristine polymer. As shown in Figure 5, the SEM analysis of the treatments suggests that the control has significantly higher levels of biofouling, compared to the other treatments, and different concentrations of CNMs/PAC. In Figure 5, Samples A2, A5, A8, and A11 demonstrate significant inhibition in bacterial growth, where the colonies were distributed scantily on the surfaces, compared to the pristine membrane. Similarly, Samples A8, A9, and A10 showed moderate levels of inhibition to the *S. aureus* colony extension and growth across the film. In these treatments, the inhibition resulted in distinctive features of avoiding cell-to-cell communication and quorum sensing ability during biofouling. However, the A3 treatment showed a converse inter-relationship with the film and other treatments. In the case of A6, the bio-film formation on the composite membrane was moderate to high; however, the colony structure and interactive behavior of *S. aureus* was disjointed by increasing the CNMs/PAC loading. The antibacterial activity of the composite and pristine membrane materials against *S. aureus* were tested by placing the membrane in liquid medium inside the flask after exposure intervals of 24 hr. The bacterial survival was estimated by successive measurements by plating and calculating in a flask.

The number of survived cells, versus the exposure time for the composite membranes, is presented in Table 4. The pristine PVDF-HFP membrane did not exhibit any noticeable antibacterial activity. In contrast, the composite membrane was affected by the incorporation of the superhydrophobic CNMs/PAC to the membrane structure by imparting its unique antibacterial feature. The results showed that the CFU for *S. aureus* significantly (*p < 0.001*) increased, as evidenced by Figure 5 (A11), after 24 hr of treatment, compared to the control sample.

SEM images in Figure 6 supported the results of cytotoxicity, where the comparison between the images shows the presence of *S. aureus* bacteria on the pristine PVDF-HFP membrane (Figure 6b), with almost no effect on the bacterial growth. Similar behavior was noticed when the PAC was exposed to the *S. aureus* (Figure 6c). In contrast, the presence of CNMs/PAC has inhibited all the *S. aureus* cells as depicted in Figure 6a, which shows the bulk of CNMs/PAC on the pristine membrane surface, where very little number of *S. aureus* cells could be seen. Figure 6d shows the strong antimicrobial effect of CNMs/PAC against *S. aureus*. Moreover, The high-resolution transmission electron microscopy (HRTEM) image shown in Figure 6e reveals the helical-shaped CNT.

3.3 | The effect of membrane surface morphology on the inhibition of cells

Membrane is a very susceptible surface, which can be affected by biofouling. Biofouling phenomenon is caused by the accumulation of microorganisms on the membrane surface creating biofilm that led to block the pores. Morphology is the most important factor mainly gained during the membrane formation process. The fabrication process of membrane can affect the hydrophobicity, roughness, pore size, and porosity.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Log CFU/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>In 24 hr</td>
</tr>
<tr>
<td>A1</td>
<td>8.99300 ± 0.03a</td>
</tr>
<tr>
<td>A2</td>
<td>8.95036 ± 0.04b</td>
</tr>
<tr>
<td>A3</td>
<td>8.89376 ± 0.12c</td>
</tr>
<tr>
<td>A4</td>
<td>8.97174 ± 0.06d</td>
</tr>
<tr>
<td>A5</td>
<td>8.92169 ± 0.02e</td>
</tr>
<tr>
<td>A6</td>
<td>8.97909 ± 0.08b</td>
</tr>
<tr>
<td>A7</td>
<td>8.93852 ± 0.06b</td>
</tr>
<tr>
<td>A8</td>
<td>8.79727 ± 0.04d</td>
</tr>
<tr>
<td>A9</td>
<td>8.81358 ± 0.05d</td>
</tr>
<tr>
<td>A10</td>
<td>8.99826 ± 0.04b</td>
</tr>
<tr>
<td>A11</td>
<td>8.91698 ± 0.07b</td>
</tr>
<tr>
<td>PVDF-HFP</td>
<td>8.76 ± 0.09b</td>
</tr>
</tbody>
</table>

Note: CFU = colony forming units; ± shows the values are the mean of five replicates. The values represent the mean of five replicates, where the different letter(s) in each column represents a significant difference (*p < 0.05*) evaluated through the dmrt test.
The hydrophobicity of the surface is generally expressed in terms of the contact angle (CA). Superhydrophobic CNMs/PAC can improve the membrane surface hydrophobicity, compared to the pristine PVDF-HFP, which is reported to have a CA of 90.1° ± 1.7°.37 The expected impact of accumulated S. aureus bacterial growth on the surface of pristine PVDF-HFP is that it can lead to irreversible adhesion of biofouling layers on the membrane. However, after adding the CNMs/PAC, the accumulation was less dependent on the amount of CNMs/PAC. The results showed that the composite membrane CA is in the range of 110° (for sample A1) to 125.6° (for sample A3), as shown in Figure 7. The SEM results revealed a decrease in the number of bacteria colonies, as well as changes in their structure, with the increase of hydrophobicity (CA). There is a decrease in S. aureus bacterial growth from 8.25527 to 7.60206 by adding CNM/PAC that led to an increase in the CA values. The inhibition effect of CA or hydrophobicity, is evident regardless of the CNM/PAC toxicity. This is supported by the fact that the CFU decreases with the increase of CA in both loading values compared to the PVDF-HFP. The synergistic effect of CNMs/PAC toxicity, as conjugated with hydrophobicity, is supported by the of adding minimum loading of CNM/PAC 7.602060 and maximum loading of CNM/PAC 8.20412 for same CA. Consequently, comparing the prepared novel composite membrane, which contains CNMs to that composed of pristine PVDF-HFP, the surface of the composite membrane, after loading high levels of CNMs/PAC, exhibited anti-biofouling characteristics. However, by increasing CNMs/PAC loading to the membrane, the bacteria were remarkably reduced. This observation is total agreement with the published studies. Such studies reported high hydrophobicity helped controlling biofouling and that the surface roughness and chemistry combination that lead to high CA were responsible for the reduction of bacteria activity against the attachment to the membrane surface.38,39

**FIGURE 6** The effect of CNMs/PAC on *Staphylococcus aureus* distribution includes (a) bulk of CNMs/PAC on pristine PVDF-HFP, (b) pristine PVDF-HFP surface, (c) PAC on membrane surface (d) Helical CNTs on the membrane surface and (e) The HRTEM image of helical CNTs. CNTs, carbon nanotubes; CNMs/PAC, carbon nanomaterials impregnated on/powder activated carbon; PVDF-HFP, poly-vinylidene fluoride-co-hexafluoropropylene; PAC, powder activated carbon

**FIGURE 7** Contact Angle of composite membrane fabricated through the design of experiment (DoE) by phase inversion.
The surface roughness plays an important role on the macroscopic characteristics of the flat sheet composite membrane. The surface roughness is affected by adding CNMs/PAC that can be clearly seen in atomic force microscope (AFM) images. The fabrication parameters affect the surface roughness of the composite membrane as shown in Figure 8. The effectiveness of CNMs/PAC on the membrane surface can deform the bacterial survival. However, high concentration of polymer with high amount of CNMs/PAC will increase the valley region between the nodule alignments on the membrane surface that results in increasing the growth of bacteria.

Figure 9 shows the effect of CNMs/PAC loading on the porosity of the fabricated membranes. With a CNMs/PAC loading of 1,000 mg, the porosity has increased up to a maximum of 88.5%. This suggests that CNMs/PAC loading plays a significant effect on the membrane porosity. Moreover, the porosity increases in the PVDF-HFP/CNMs/PAC composite membranes. This is closely linked to the prevalence and interactions of the embedded CNMs/PAC material within the polymer structure. Such construction was necessary to hinder the NMP/water exchange through the membrane pore size being one of the most effective factors for the bacterial growth. In case of tested membranes, it increased as the CNMs/PAC content was increased up to 1,000 mg, as shown in Figure 10. The best choice for MD pore size, according to the pertinent literature, is 0.2–0.5 μm.

There is an interest to understand the effect of biofouling on membrane as discussed in details below and shown in Table 5. Gryta et al. calculated the growth of bacteria (Pseudomonas and Streptococcus faecalis) on the membrane surface. They observed the biofilm on the membrane surface at the feed side and some bacterial species were passed through the membrane to the permeate side through the pores of membrane. Krivorot et al. examined the parameters influencing the biofouling formation on surface of the membrane such as pore size, porosity and roughness. Anne Bogler and Edo Bar-Zeev discussed the effect of the membrane type and the operation studies on the Anoxybacillus sp. Farid et al. studied the effect of CNTs on the bacterial growth.

3.4 | Optimization of membrane preparation parameters

Synthesis conditions of the composite membranes by phase inversion method was optimized toward the desired bacterial inhibition. The optimization used the obtained mathematical-statistical model of the RSM procedure. The goal for adding superhydrophobic CNMs/PAC is to minimize the number of the bacterial colonies. For economic purposes, the optimization criterion was to maximize the inhibition with
minimal quantities of CNMs. At the same time, the polymer concentration should be kept in the desired range with minimal membrane thickness. The model predicted multiple sets of combinations and then the best set was selected as the optimum based on the degree of desirability. The results were ranked by desirability of the given model. The optimum preparation conditions of the composite membrane providing the highest desirability were carbon loading CNMs/PAC of 363.04 mg, concentration of PVDF-HFP 22.64 g/100 g solvent, and a thickness of the casting knife of 133.91 μm. These conditions provided low survival values of S. aureus of about 7.56229 CFU.

**TABLE 5** Some studies on biofouling on membrane surface

<table>
<thead>
<tr>
<th>Foulant</th>
<th>Membrane type</th>
<th>Pore size (μm)</th>
<th>Porosity (%)</th>
<th>Observation</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pseudomonas &amp; Streptococcus faecalis bacteria</td>
<td>Capillary PP</td>
<td>0.22</td>
<td>73%</td>
<td>Bacterial growth was obvious on the membrane surface</td>
<td>42</td>
</tr>
<tr>
<td>Marine bacteria</td>
<td>Hollow Fiber PP</td>
<td>0.6</td>
<td>70%</td>
<td>The flux experiment slight decline was observed after 180 hr</td>
<td>43</td>
</tr>
<tr>
<td>Bacteria</td>
<td>Flat-sheet PVDF</td>
<td>0.22 or 0.2</td>
<td>80%</td>
<td>The submerged membrane distillation bioreactor process used to increase the flat sheet membrane flux with long life</td>
<td>44</td>
</tr>
<tr>
<td>Lactobacillus casei, Lactococcus lactis, and Leuconostoc mesenteroides bacteria</td>
<td>Capillary PP</td>
<td>0.22</td>
<td>73%</td>
<td>The influence of biofouling film is causing a decline of the permeate flux by 50%.</td>
<td>45</td>
</tr>
<tr>
<td>Anoxybacillus sp. bacteria</td>
<td>EPS</td>
<td>0.45</td>
<td>-</td>
<td>The growth of bacteria is declined 78% of distillate water flux</td>
<td>46</td>
</tr>
<tr>
<td>Escherichia coli bacteria</td>
<td>CNTs + PVDF</td>
<td>0.45</td>
<td>-</td>
<td>The composite membrane has declined the biofilm on membrane and increase the water flux with high salt rejection</td>
<td>47</td>
</tr>
</tbody>
</table>

**FIGURE 11** Cross-section and atomic force microscope (AFM) images of the top surface of the optimum composite membrane fabricated by phase inversion method
The composite membrane was prepared later according the optimum parameters of optimum values obtained from RSM—DOE for further investigation and applications. Figure 11 shows the SEM images of the composite membrane at the top figure-like structure and at the bottom layer sponge-like structure. The AFM images exhibited high average roughness (43.4) with less aggregate found at the membrane surface.

4 | CONCLUSIONS

A novel superhydrophobic surface nanocarbon-based membrane was fabricated following the DoE and CCD technique to optimize the parameters of the membrane preparation procedure for minimum bacterial growth as the response and optimization objective function. The membrane was successfully prepared using the phase inversion method. Adding CNMs/PAC to the composite membrane resulted in a high CA as well as noticeable superiority against biofouling formed by *S. aureus* bacteria. It was found that the incorporation of superhydrophobic CNMs/PAC did not only increase CA but it also enhanced the anti-adhesive characteristics and antibacterial properties; conditions required for the MD application. The optimum membrane preparation conditions for the highest desirability were carbon loading CNMs/PAC of 363.04 mg, concentration of PVDF-HFP 22.64 g/100 g solvent, and a thickness obtained of the casting knife gap of 133.91 µm. These parameters have afforded the lowest survival values of *S. aureus* of about 7.56229 CFU.

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