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Fabrication, tuning and performance analysis of polyacrylonitrile (PAN)-derived microfiltration membranes for bacteria removal from drinking water

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Abstract−Removal of bacterial contaminations from water using advanced technologies is one of the essential steps in improving human health. The present study's aim was to develop high performance microfiltration membranes from polyacrylonitrile (PAN) for bacteria removal from drinking water. The characteristics and performance of membranes were tuned through exploring the variation of prominent fabrication and operating parameters. The findings reveal that increasing PAN concentration in dope and addition of citric acid were successful in tailoring membrane microstructure. Bacteria rejection in modified membranes improved by exhibiting high log removal values (LRV) ranging from 3.92 (99.87%) to 5.57 (99.99%) while permeate fluxes were in the range of 35.83-58.62 $L\cdot m^{-2}\cdot h^{-1}$. The trends are explained by taking into account the structural characteristics of bacterial strains. Exploring the effect of operating parameters on the performance of membranes revealed that increase in feed concentration from 10^3 to 10^7 $cfu\cdot ml^{-1}$ improved membrane rejection. The largest rejection (5.57) was observed toward *Staphylococcus aureus* at feed concentration of 10^7 $cfu\cdot ml^{-1}$. Similarly, rejection improved upon reducing operating pressure from 3.5 to 1.5 bar. Also, shifting of feed pH to 7.4 and 9.4 enhanced membrane rejection to as high as 4.60 and 5.66 toward *E. coli* and *Staphylococcus aureus*, respectively. This was attributed to the zeta potential and isoelectric point values of the membranes and involved strains. Overall, the findings revealed that the developed PAN membranes are more effective in removal of gram-positive strains and their rejection is strongly dependent on the peptidoglycan layer of strains.

Keywords: Microfiltration Membrane, Polyacrylonitrile, Bacteria Removal, Drinking Water, Rejection Performance

INTRODUCTION

Membrane technology has emerged as a popular technique for the treatment of various liquid and gas streams with diverse industrial applications [1-7]. The lack of quality water in some parts of the world as well as the importance of water reuse in parallel with the strict regulations have created immense motivations for development of reliable membrane processes for this purpose [8,9]. Among the few types of membrane processes, microfiltration (MF) membranes have shown promising results for the treatment of waters contaminated with microorganisms such as bacteria and viruses.

In recent years, and upon large entry of urban wastewater into groundwater resources, the number of diseases caused due to the contamination of drinking water with bacteria has increased. According to the World Health Organization, the mortality rate because of the use of bacteria contaminated water has been shockingly high with more than 5 million lost lives only in 2008 [10]. The majority of the mortalities were attributed to the drinking of water contaminated by a group of bacteria including *Staphylococcus*, *Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeruginosa* and *Enterococcus faecalis*. In 1986, the infections associated with the above group of bacteria accounted for 84.9% of all cases of food poisoning in the United States. This record increased to more than 95% in 1991.

Among the diverse range of microorganisms, bacteria fall in the mid-range size between 0.5 to 10 μm. On the other hand, the size of the pores in commercial MF membranes is typically between 0.1 to 1.0 μm. While these pores are so small and expected to remove all the bacterial strains, but in many cases bacteria have been able to pass through and be found in the permeate streams. This is largely due to the irregular and asymmetric shape of the bacterial species which enables them to pass through. Thus, it is always advised to design membrane systems to ensure removal efficiency complying with the standards set for each bacteria.

Polyacrylonitrile (PAN) as an engineering polymer has been used in the form of membrane to remove bacteria, viruses and other microorganisms from water. PAN, due to its special characteristics, has become one of the most popular materials in the fabrication of diverse ranges of membranes [11,12]. In addition, due to its high hydrophilicity, PAN has demonstrated reasonable resistance against common fouling in water treatment applications. The polar nature of PAN has enabled unique properties such as stability and mechanical strength, resistance to some chemicals like chlorine and other cleaning agents as well as microorganisms.

According to the available reports, PAN-based membranes have

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shown acceptable performance in the intended applications. However, very limited reports are available on the development of PAN based membranes for bacterial removal from water. Lohokare et al. [13] demonstrated that UF membranes derived from PAN having different solvents and additives showed promising results for *E. coli* removal from water. They realized that merely increasing polymer concentration does not necessarily reduce pore size of membranes. Among the investigated solvents used for the dope solution preparation, N-methyl pyrrolidone offered membranes with optimal combination of flux and rejection of various solutes. Also some of the membranes containing citric acid, tartaric acid or maleic acid offered 1.2-1.7 times higher flux than the membrane prepared using inorganic salt, $ZnCl_2$ as an additive. In another study, Kobayashi et al. [14] developed PAN ultrafiltration (UF) membranes and investigated the effect of the presence of charge groups on the rejection performance. They reported that with the increase of the polymer concentration in the DMSO cast solution, the molecular size exclusion effect of the resultant UF membrane decreased. For UF experiments of *E. coli* suspension solution with 10^7 colony forming unit/unit volume, the permeability of the bacteria through the membrane was in the range of about 10^{-3}% in PAN membranes. It was also found that *E. coli* permeation through copolymer UF membranes with charge groups was completely restricted. However, similar to the former study, the performance analysis in this study was only limited to the investigation on *E. coli*.

The main objective of the present research study was to develop high performance MF membranes from PAN for bacteria removal from drinking water. The characteristics and performance of the membranes are tuned through exploring variation of prominent fabrication and operating parameters. The intention was to improve the microstructure and morphology of the membranes through optimization of polymer concentration and addition of an effective additive to the dope to obtain high performance rejection for bacteria removal. On the other hand, the effect of key operational parameters, such as concentration of bacterial strains in feed, operational pressure and pH, was investigated to further enhance the separation performance. To the best of our knowledge, this is the first systematic study on the development of MF membranes from a commercial polymer for the treatment of drinking water contaminated with bacteria. Another specific feature of this research is consideration of diverse ranges of bacterial strains to gain insight about the role of their characteristics on the removal efficiency. It was expected that the outcomes would provide valuable insight for design, formulation and fabrication of high performance MF membranes for improving water quality.

EXPERIMENTAL

1. Materials

PAN (M_W=90,000 g·mol^{-1}, density: 1.184 g·cm^{-3}, CAS No. 25014-41-9) with chemical formula of $(C_3H_3N)_n$ was supplied by Polyacryl Co. (Isfahan, Iran) in the form of white powder. Dimethylsulfoxide (DMSO) was procured from Dae-Jung (South Korea) and used as the solvent for preparation of dope solutions. Citric acid (M_W=192.12 g·mol^{-1}, density: 1.665 g·cm^{-3}, CAS No. 77-92-9) in the form of white color granules obtained from Merck (Germany). Brain Heart Infusion (BHI) broth and Nutrient Agar (NA) were procured from Merck and used as bacteria culture media. All the chemicals were used as received.

2. Preparation of Asymmetric PAN MF Membranes

First, PAN powders were dried in a vacuum oven at 60 °C for 24 h. For the preparation of the dope solutions, PAN powders were gradually dissolved in DMSO in two concentrations of 15 and 17 wt% while stirring at 600-700 rpm. The temperature was controlled at 70 °C in the course of dissolution. Stirring lasted for 24-36 h until homogeneous solutions were obtained. To tune the membrane microstructure, additional solutions were also prepared by adding 4 wt% citric acid (as an organic acid additive) to the dope and the effects were investigated.

Flat sheet MF membranes were prepared following the non-solvent induced phase separation [15]. Accordingly, all dope solutions were cast on a glass plate by using an automated casting machine at a knife gap of 200 μm. The phase inversion was initiated upon immediate immersion of the cast films into the coagulation bath filled with deionized (DI) water at the controlled temperature of 40 °C. The nascent films were stored in water for at least an overnight to ensure completion of phase inversion process and removal of residual solvents [16]. Membranes were labeled in the format of M-X-Y in which M denotes membrane, and X and Y stand for the concentrations of PAN and citric acid in the dope solutions, respectively. For example, M-15-4 represents the membrane prepared from the dope solution containing 15 wt% PAN and 4 wt% citric acid.

3. Preparation of Bacteria and Bacterial Feed Suspensions

All the tests were performed on five diverse bacterial strains and a mixture of them as follows: *E. coli*, *Salmonella typhi*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Enterococcus faecalis*, all supplied by Pasteur Institute of Iran. The selection of these bacterial strains was based on their diverse morphological and structural characteristics, including size, shape and gram staining. In addition, the selected species are easy to cultivate since they do not need any specific atmosphere or media for growth. Also, their gen-

Table 1. The morphological and structural characteristics of the bacterial strains used in this research

Bacterial strain	Gram	Shape	Mean size (μm)	Smallest width-length (μm)	Cell volume (μm^3)	Isoelectric point	Optimum pH for growth	Ref.
E. coli	–	Bacilli	0.5×2	0.6-2	0.508	2.70	6.5-7.5	[17]
Salmonella typhi	–	Bacilli	1.5×2	0.7-2	0.679	2.12	6.5-7.5	[18]
Staphylococcus aureus	+	Coccus	0.8	0.7-0.7	0.179	1.90	7-7.5	[19]
Pseudomonas aeruginosa	–	Bacilli	1.6×1.8	0.5-1	0.163	2.17	7.2-7.6	[20]
Enterococcus faecalis	+	Coccus	0.8	0.6-0.6	0.113	2.40	7-7.5	[21]

eration time is relatively short and this enables obtaining results after overnight incubation. Specifically, *E. coli* is a fecal indicator with well-known characteristics systematically checked in drinking water. The morphological and structural characteristics of the bacterial strains used in this study are provided in Table 1.

The performance of the membranes was evaluated using feeds prepared from each bacteria strain as well as a bacterial mixture containing all five strains with specifications listed in Table 1. For determination of the concentration of each feed, optical density measurement was conducted at time intervals of 1, 2, 4 and 6 h after addition of each bacterium to the culture cell. According to the instructions provided by the supplier, certain amounts of BHI broth were weighed and then added to the bottles containing 30 and 300 ml of DI water followed by rigorous shaking to ensure complete dissolution. It was also essential to ensure no BHI broth residuals remained on the bottle walls. The containers were then sterilized by autoclaving at 121 °C for 15 minutes. The containers then remained in the autoclave for natural cooling. In a microbial hood, a limited number of the colonies cultivated from each bacterium were added to a 30 ml suspension for inoculation. The container was then incubated at 37 °C while shaking at 250 rpm for 16 h. Afterwards, 3 ml of each suspension was added to a 30 ml of a new broth nutrient suspension and incubated at 37 °C while shaking at 250 rpm for 2-4 h. The incubated suspension was used for determining concentration by using optical density measurements in a spectrophotometer at the wavelength of 610 nm. About 3 ml of the incubated suspension was diluted by transferring into a 300 ml of new broth nutrient in order to achieve the desired concentration. Fig. 1 demonstrates the steps involved in the preparation of feeds.

4. Concentration Evaluation

Dilution is one of the basic methods for determining the concentration of bacterial systems [22]. In this method, a certain volume of the bacterial suspension is prepared and then 0.1 ml of it is added to 0.9 ml of BHI broth in a microtube in order to achieve 1/10 dilution. Similar procedure was used for the preparation of other dilutions at the levels of 1/1,000, 1/10,000, 1/20,000, 1/50,000 and 1/100,000. Then, 0.01 ml from each dilution was transferred to the plates containing solid culture cells (nutrient agar) and well distributed. For better cultivation, plates were placed in an incubator at 37 °C for at least 16 h.

Colony forming units (CFU) were enumerated after overnight incubation of the plates at 37 °C. Accordingly, the bacteria growth at each plate was counted and then the corresponding bacteria concentration was calculated using Eq. (1) [13]:

$$\mathrm{CFU/ml} = N_c \cdot D_f / V_{c.p} \quad (1)$$

in which N_c is the number of counted colonies, D_f is the dilution coefficient, and $V_{c.p}$ is the volume of the culture used. All the steps involved were carried out inside a microbial hood using sterilized glass wares. The same procedure was used for determination of the concentration of bacteria in feed and permeate streams during each test. In other words, bacterial viability and cultivability were controlled during the filtration test by evaluating the concentration of the feed suspension before and after each test.

5. Experimental Setup and Testing Procedure

Performance evaluation of the membranes was accomplished in a cross-flow filtration set-up schematically shown in Fig. 2 [23]. This setup was equipped with a flat cell comprised of an inlet for the feed and two outlets for permeate and retentate streams. During the experiments, the retentate stream always returned to the feed tank in order to control the concentration of the feed close to its initial level. The trans-membrane pressure was controlled by a valve located at the retentate stream. The internal units of the set-up were

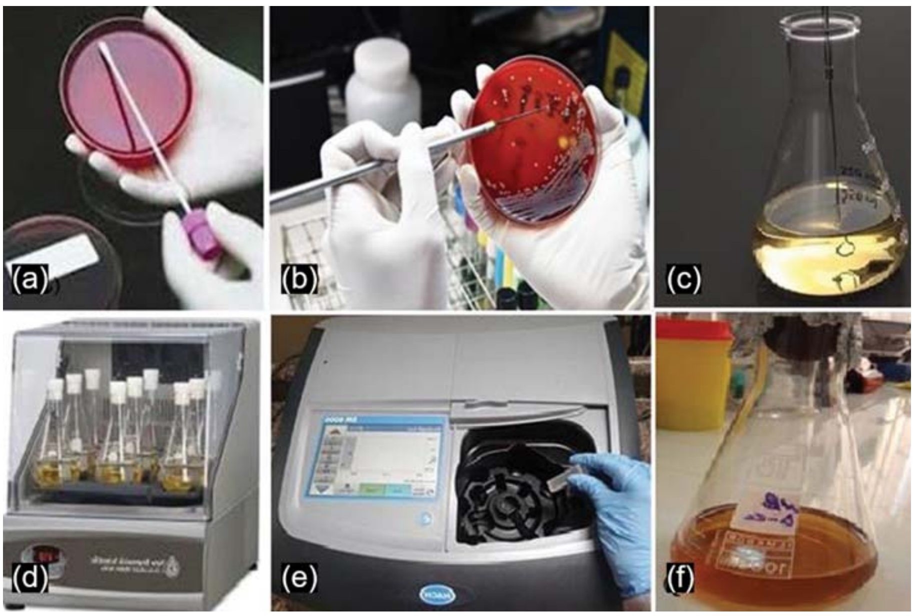

Fig. 1. Steps involved in the preparation of feed suspensions containing bacteria. (a) Cultivation of bacteria on the solid culture cell, (b) and (c) Transfer of colonies from solid to liquid culture cell, (d) Transfer of liquid culture cell to the incubator, (e) Optical density measurement, (f) Dilution of 0.01 and preparation of final desired concentration.

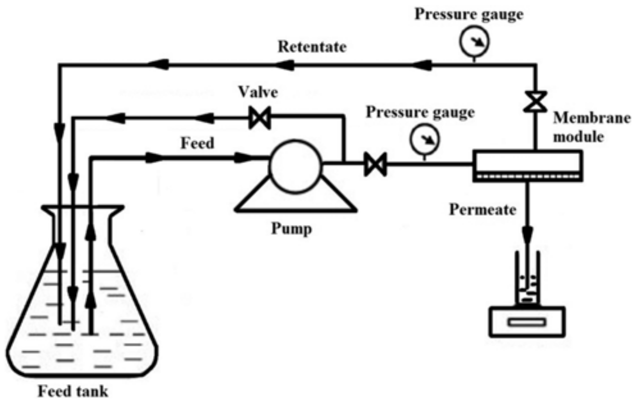

Fig. 2. The schematic of the cross-flow MF setup.

sterilized by circulating a solution containing 10 wt% sodium hypochlorite for 20 minutes followed by rinsing with distilled water.

Before mounting, membrane samples were cut in the size of approximately 6 cm^2 and sterilization was performed similar to the prescribed method by immersion in the solution containing 5 wt% sodium hypochlorite for 10 minutes followed by rinsing with distilled water.

The pure water flux (PWF) for each membrane was obtained by using DI water as the feed and data were collected after 20 minutes at stable conditions and at room temperature. For the measurement of permeate flux in the presence of feed containing bacterial strains, the membranes were sterilized and examined for their performance using feeds containing certain concentrations of bacteria at the trans-membrane pressure of 1.5 bar and flow rate of 10^7 cfu·ml^{-1}. The PWF and permeate flux (J_w) in L·m^{-2}·h^{-1} were calculated based on the total volume of permeate collected over time using Eq. (2) as follows [24]:

$$J_w = \frac{\Delta V}{A \cdot \Delta t} \qquad (2)$$

where ΔV is the quantity of permeate (L), A is the membrane active area (m^2), and Δt is the sampling time (h). The rejection performance of the membranes was calculated based on log removal value (LRV) according to the Eq. (3):

$$LRV = \log (C_f/C_p) \qquad (3)$$

in which C_f and C_p are the bacterial concentrations (cfu·ml^{-1}) in the feed and permeate, respectively. According to Eq. (3), an LRV of 1 is equivalent to 90% removal of the target strain, an LRV of 2 is equivalent to 99% removal and an LRV of 3 is equivalent to 99.9% removal and so on.

The effect of feed concentration was investigated in the range of 10^3, 10^5, 10^7 cfu·ml^{-1} for all the bacterial strains at the pressure of 1.5 bar. In addition, the effect of feed pressure on the performance of the membranes was investigated by testing at three trans-membrane pressures of 1.5, 2.5 and 3.5 bar. The effect of pH was investigated on two representative gram-positive and gram-negative bacterial strains at pH range of 5.4, 7.4 and 9.4. All the experiments were repeated at least three times to ensure reproducibility of the results.

6. Characterization

The porosity (ε) of membranes was calculated by the following equation:

$$\varepsilon = \frac{W_s - W_d}{A \cdot L \cdot \rho} \qquad (4)$$

where W_s and W_d denote the weights of a membrane at swelling and dry states, respectively; A is the membrane area; L is the average thickness of the membrane and ρ is the water density. Also the mean pore size was calculated using Eq. (5) as follows:

$$r_m = \sqrt{\frac{(2/9 - 1/75\varepsilon) 8 \eta LQ}{\varepsilon A \rho}} \qquad (5)$$

The cross-sectional morphology and microstructure of membranes were examined using scanning electron microscopy (SEM). Dry membrane samples were frozen in liquid nitrogen and then fractured. Samples were coated with gold prior to examination.

The cell volume for each bacteria was calculated based on the principle of a rod-shaped particle with round ends using the following equation [17]:

$$V = \frac{4}{3}\pi r^3 + \pi r^2 (L - 2r) \qquad (6)$$

where V is the cell volume (μm^3), r (μm) represents the half of the smallest width and L (μm) represents the length of the bacterial cell.

RESULTS AND DISCUSSION

1. Analysis of the Bacterial Growth

The first step in the present study was to investigate and ensure the growth for the bacterial strains. For this purpose, all the selected

Table 2. The results of optical density (OD) measurements and corresponding growth for the bacterial strains

Time (h)	1		2		4		6	
Strain	OD	cfu·ml^{-1}	OD	cfu·ml^{-1}	OD	cfu·ml^{-1}	OD	cfu·ml^{-1}
E. coli	0.39	>1,000	0.48	4 E+5	1.10	4 E+7	1.39	3 E+8
Salmonella typhi	0.37	>1,000	0.58	7 E+5	1.26	2 E+8	1.40	8 E+8
Staphylococcus aureus	0.41	>1,000	0.70	1 E+6	1.67	1 E+9	2.19	4 E+9
Pseudomonas aeruginosa	0.26	>1,000	0.32	2 E+4	0.74	3 E+6	1.12	1 E+9
Enterococcus faecalis	0.35	>1,000	0.53	3 E+5	1.47	7 E+8	1.94	5 E+9
Bacterial mixture	0.37	>1,000	0.56	2 E+5	1.28	6 E+7	1.83	2 E+9

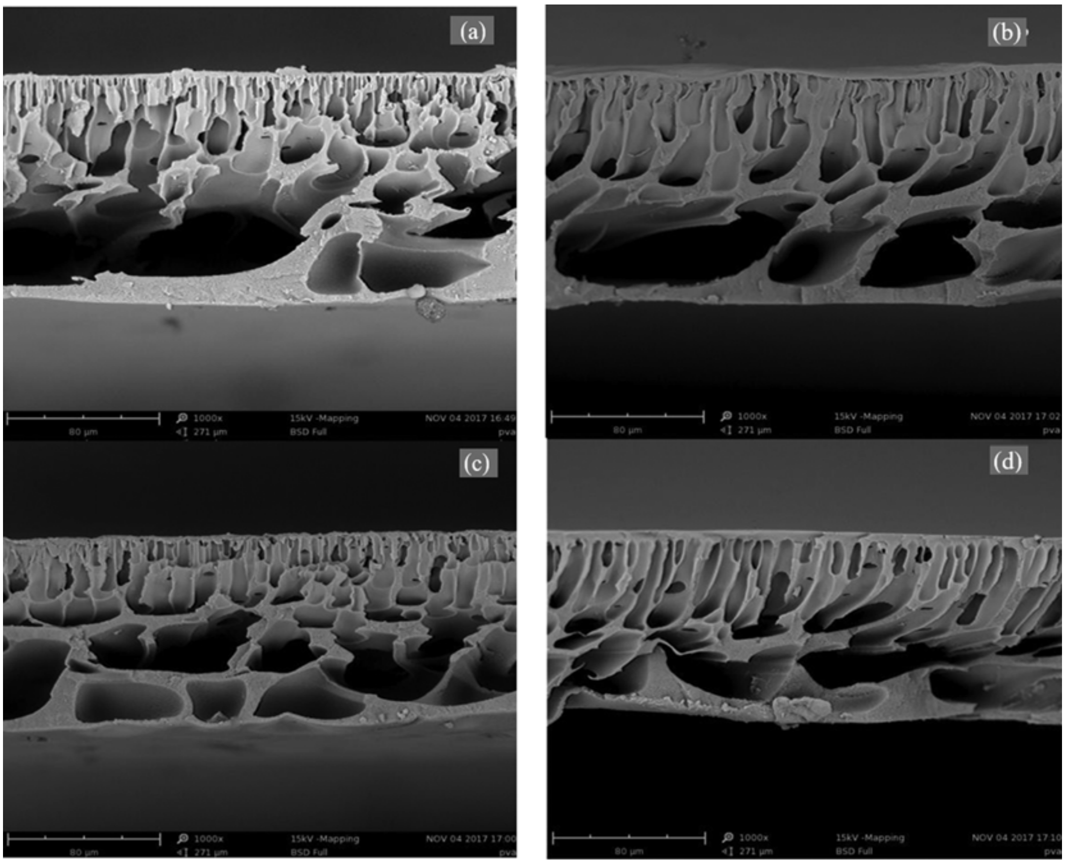

Fig. 3. The SEM cross-sectional morphology of the membranes demonstrating the effect of polymer concentration and addition of citric acid: (a) M-15-0; (b) M-17-0; (c) M-15-4; (d) M-17-4.

bacterial strains were cultured in BHI broth. Then, optical density measurements were carried out using a spectrophotometer at the different time intervals. Table 2 shows the optical density values and corresponding growth for each bacterium. The results indicate that for all the bacterial strains and the mixture concentration of at least 1,000 cfu·ml^{-1} was achieved. However, the rate was different for the second h and after 4 and 6 h and indicating that the adopted procedure was successful in providing feeds with appropriate concentrations for fulfillment of experimental requirements.

2. The Effect of Polymer Concentration on the Characteristics and Performance of Membranes

It is well known that the concentration of polymer in the dope solution has great effects on the characteristics and morphology of the resultant membranes through its contribution in the phase inversion process [25]. Accordingly, membranes were prepared by two dopes containing 15 and 17 wt% PAN in DMSO in order to investigate and compare the results. Fig. 3(a) and (b) show the cross-sectional morphology of the membranes M-15-0 and M-17-0 containing no citric acid, respectively.

It can be seen that both dope solutions offer asymmetric structures comprised of a thin skin layer on the top and a sponge-like structure full of large macrovoids beneath. Essentially, the formation of macrovoids in the microstructure of the MF membranes is very typical due to the relatively low concentration of the polymer which should be used in accordance with the three-phase diagram to prevent formation of dense skin layer. Hamzah et al. showed that such trend is due to the rapid rate of non-solvent inflow compared to solvent outflow [26]. Comparison of the morphologies revealed that population of macrovoids at the close vicinity of the skin layer was far more in M-15-0 than in M-17-0. This trend

Table 3. The morphological characteristics of MF membranes

Membrane	Citric acid (wt%)	Membrane thickness (μm)	Porosity (%)	Mean pore size (μm)
M-15-0	0	141±5	84.36	0.41±0.01
M-17-0	0	154±6	79.57	0.32±0.01
M-15-4	4	126±5	75.49	0.35±0.01
M-17-4	4	137±4	68.11	0.25±0.01

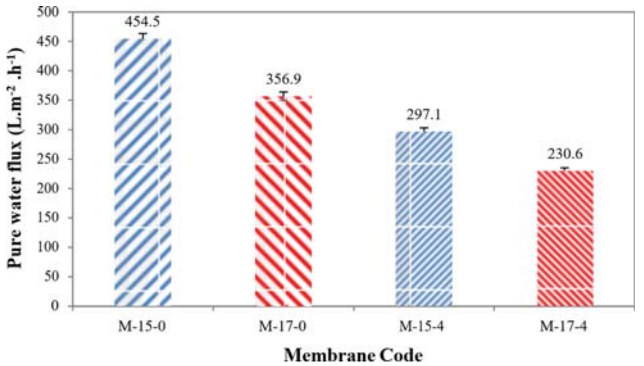

Fig. 4. The pure water flux (PWF) data for the developed membranes showing the effect of polymer concentration and citric acid in dope solution (P=1.5 bar).

could be attributed to the increased viscosity of the M-17-0 dope solution and its effect on suppressing the rate of intrusion of water molecules as coagulating agent during the demixing with DMSO molecules. It is well demonstrated that the reduction in the rate of demixing led to the formation of a thicker skin layer with fewer macrovoids in the cross-section [27]. A similar trend was reported by Lohokare et al. in the case of PAN in which the membranes prepared from the dope with higher polymer concentration offered a thicker skin layer and a support layer with less porosity [13].

The microstructure of the membranes was further analyzed by comparing the porosity and mean pore size. According to the data in Table 3, it was noted that the porosity of the membranes reduced from 84.36% to 79.57% upon increase in PAN concentration in the dope from 15 to 17 wt%. Similarly, mean pore size values reduced from 0.41 to 0.32 µm.

The results of analysis of PWF for the membranes are shown Fig. 4. It was noted that PWF for the membrane prepared from 15 wt% PAN in the dope was 454.5 $L\cdot m^{-2}\cdot h^{-1}$. However, PWF decreased to 356.9 upon increase in PAN concentration to 17 wt%, which is equivalent to about 21%. These results are in good agreement with the data in the Table 3 which showed reduction in porosity and mean pore size. In addition, this can partially be related to the slight increase in the overall membrane thickness from 141 to 154 µm. According to Kim et al., the use of higher polymer concentration led to the formation of a thicker skin layer and closed cell pores in the cross-section [28]. Comparison of the SEM images in Fig. 3(a) and (b) can easily confirm the presence of slightly thicker skin layer in M-17-0 membrane compared to M-15-0. However, no judgment could be made about the structure of the pores below the skin layer. Similar effects of polymer concentration on the morphology and performance of the membranes are reported elsewhere [29,30].

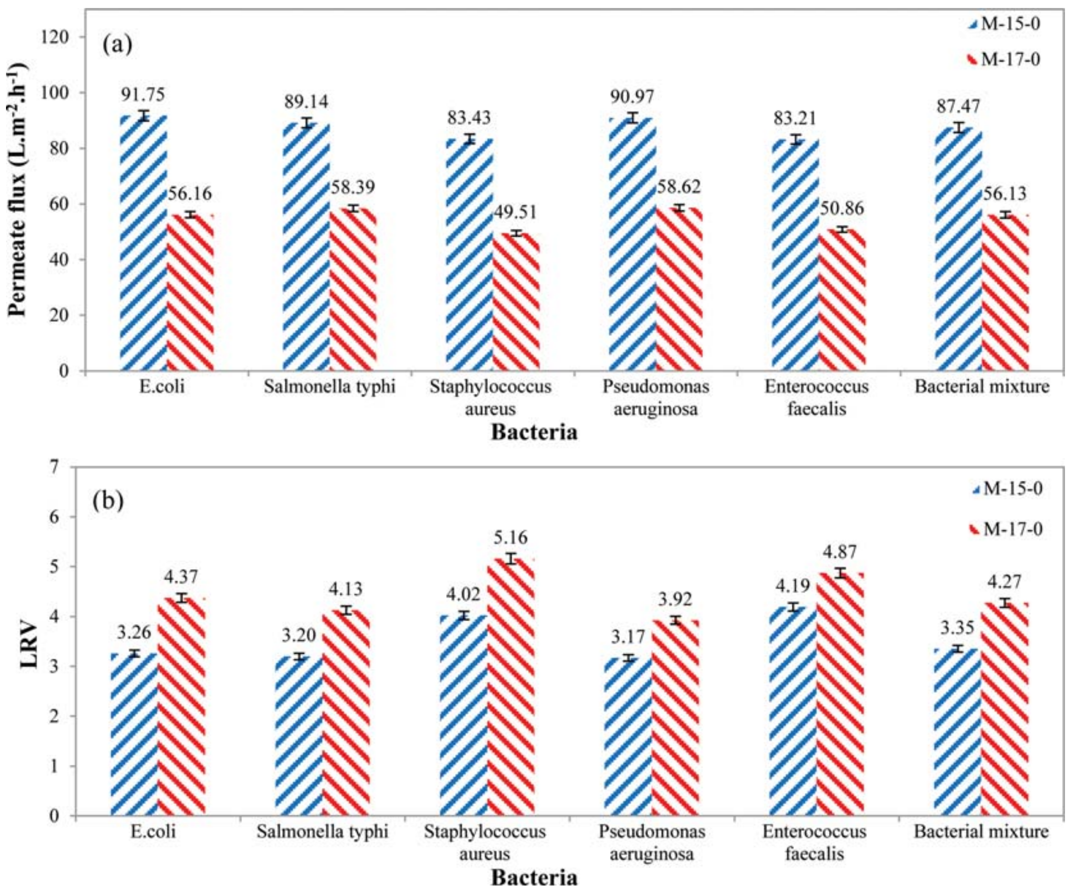

Fig. 5. The effect of polymer concentration in dope solutions on (a) permeate flux and (b) rejection of membranes. (Operating conditions: P=1.5 bar, pH=7.4, Feed conc.=10^7 $cfu\cdot ml^{-1}$).

The performance data for the membranes are shown in Fig. 5. It can be seen from the trends in Fig. 5(a) that all M-15-0 membranes exhibited considerably higher permeate flux ranging from 83.21 to 91.75 $L\cdot m^{-2}\cdot h^{-1}$ compared to M-17-0 membranes. In contrast, the permeate flux in M-17-0 membranes was limited to the range of 49.51 to 58.62 5 $L\cdot m^{-2}\cdot h^{-1}$. Essentially, the permeate flux values were considerably lower than the PWF data obtained for these membranes, and this can be attributed to the presence of strains in the feed as well as distinct process conditions compared to the use of DI water for PWF measurements. However, the trend observed in the case of permeate flux was similar to the one observed in PWF experiments. The variations in the permeate flux for different feeds can be attributed to the role of presence of each strain in governing membrane flux.

Fig. 5(b) shows the effect of polymer concentration on the rejection of membranes. It can be seen that the membrane prepared from 15 wt% PAN offered rejections in the range of LRV:3.17 (in the case of *Pseudomonas aeruginosa*) and LRV:4.19 (in the case of *Enterococcus faecalis*). Increase in the polymer concentration to 17 wt% offered membranes with improved rejection in the range of LRV:3.92-5.16. Particularly, the rejection for *Pseudomonas aeruginosa* increased by 23.7% to LRV:3.92, while membrane rejection for *Enterococcus faecalis* increased by 16.2% to LRV:4.87. The variation in the rejection of the membranes toward the studied strains can be related to the morphology and microstructure of the membranes as well as the size and shape of the strains. The order of improvement in membrane rejection was as follows: *E. coli* (34%)> *Salmonella typhi* (29%)>*Staphylococcus aureus* (28.4%)>*mixture* (27.5%)>*Pseudomonas aeruginosa* (23.7%)>*Enterococcus faecalis* (16.2%). The findings suggest that the membranes performed the best with maximum removal of *Staphylococcus aureus*.

3. The Effect of Addition of Citric Acid to Dope Solution on the Characteristics and Performance of Membranes

In addition to the variation in polymer concentration, another effective method for tuning the membrane morphology and microstructure is through incorporation of additives. Typically, additives are added to the dope solution to act during phase inversion. The structure of the upper and lower layer of the membrane partly depends on the rate of precipitation in coagulation bath [31]. Accordingly, the type and concentration of the additive play a very important role in improving the properties of membranes. Among the diverse range of additives, citric acid as an organic acid has been an effective additive with successful results [32,33]. On this basis, citric acid was used to investigate how it can affect the property and performance of the resultant membranes.

Fig. 3 shows the SEM images for the membranes derived from the dopes with and without citric acid. Essentially, no distinct differences can be identified in the macroscopic views and it was

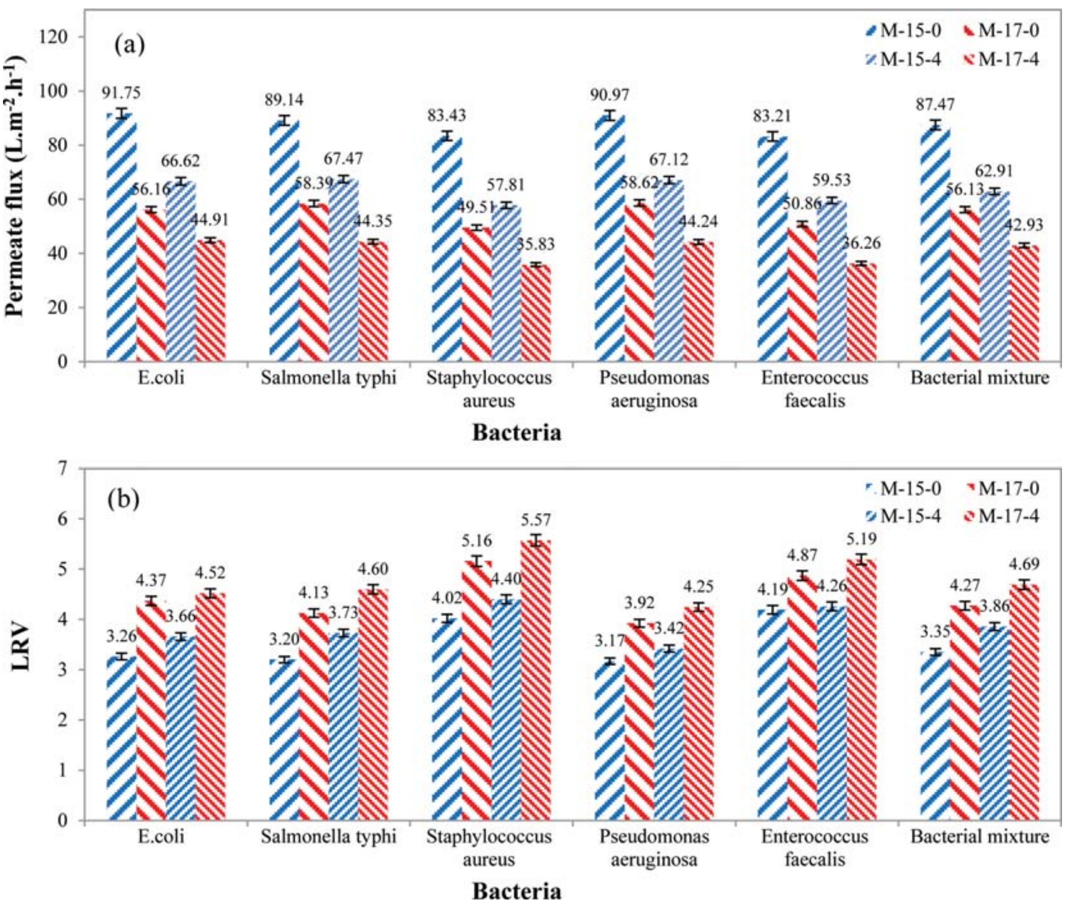

Fig. 6. The effect of addition of citric acid to dope solution on (a) permeate flux and (b) rejection of membranes. (Citric acid content: 4 wt%, Operating conditions: P=1.5 bar, pH=7.4, Feed conc.=10^7 cfu·ml^{-1}).

decided to track the microscopic variations by measuring PWF values. The effect of addition of citric acid on PWF of membranes is shown in Fig. 4. It can be seen that upon addition of 4 wt% citric acid, PWF considerably decreased for both membranes regardless of the polymer concentration. The intensity of PWF reduction for M-15-4 and M-17-4 membranes compared to their intrinsic rivals was 21.5% and 22.4%, respectively. Note that each citric acid contains molecule three carboxylic acid (-COOH) and four hydroxide (-OH) functional groups in its chemical structure, and this offers high hydrophilic power. Accordingly, the extended hydrophilic characteristic of the dope containing citric acid facilitates intrusion of water molecules upon phase inversion and, on this basis, it is expected to result in formation of more and larger macrovoids. Ghaemi et al. confirmed this and increased PWF upon addition of 0.75 wt% citric acid in the case of polysulfone membranes [34]. However, the same study and another study by Alaei et al. [32] showed that increase in citric acid concentration beyond a certain level disrupted this trend and reduced PWF. According to the other findings, the unexpected shift in trend could be attributed to the formation of strong complexations between the molecules of solvent DMSO and citric acid and thus increase the viscosity of dope solution [13]. The increased viscosity of the dope solution in fact plays the same role as increase in polymer concentration through promoting delayed demixing. This competing effect could overcome the effect of hydrophilicity at higher concentrations of citric acid [32,34]. Data in Table 3 also confirms that the porosity and mean pore size for M-15-4 and M-17-4 membranes were less than their corresponding M-15-0 and M-17-0 membranes, respectively.

The performance data for the membranes are shown in Fig. 6. It can be seen from the trends in Fig. 6(a) that for the membranes prepared from either 15 or 17 wt% PAN in the dope, permeate flux reduced upon addition of citric acid. While permeate flux value for M-15-0 membranes was in the range of 83.21-91.75 $L\cdot m^{-2}\cdot h^{-1}$, the range for M-15-4 membranes was 57.81-67.47 $L\cdot m^{-2}\cdot h^{-1}$. Similarly, permeate flux for M-17-0 membranes was in the range of 49.51-58.62 $L\cdot m^{-2}\cdot h^{-1}$ and for M-17-4 membranes in the range of 35.83-44.91 $L\cdot m^{-2}\cdot h^{-1}$. Although addition of citric acid had negative effects on permeate flux, desirably improved effects was noticed in terms of rejection. According to Fig. 6(b), the rejection of the membranes derived from the dopes containing citric acid toward all the investigated bacterial strains improved considerably. While the rejection for M-15-0 membranes was in the range of LRV:3.17-3.73, rejection of M-15-4 membranes improved to LRV:3.42-4.40. Similarly, the rejection in M-17-0 membranes was in the range of LRV:3.92-5.16, and improved to LRV:4.25-5.57 for M-17-4 membranes. The best rejection was obtained in M-15-4 and M-17-4 membranes toward *Staphylococcus aureus* by offering LRV:5.16 and LRV:5.57, respectively. These findings clearly demonstrate the successful application of the use of citric acid in dope formulation for tailoring membrane microstructure and rejection performance. Of note is that microorganisms and bacteria are negatively charged at pH values above the isoelectric point, while pH values less than the isoelectric point are positively charged [35]. According to the data in Table 1, all five bacterial strains have isoelectric points between 1.9 and 2.7 and this reflects their negative charge in natural water pH. The negative charges on the surface of bacteria and membrane lead to the electrostatic repulsion between and leads to increased rejection of bacteria along with size exclusion as the primary mechanism.

4. The Effect of Characteristics of Bacterial Strains on the Membrane Performance

The main impetus in this research was to develop membranes with desirable properties for effective removal bacteria from water. To examine the features of developed membranes, diverse ranges of bacterial strains having distinct characteristics were selected. According to the data in Table 1, *E. coli*, *Salmonella typhi* and *Pseudomonas aeruginosa* are all bacilli in shape, whereas *Staphylococcus aureus* and *Enterococcus faecalis* are spherical. Considering the larger size of the bacterial strains compared to the pore dimensions of the membranes, it was anticipated that all the membranes could offer complete rejection. However, it was noticed that bacterial strains were present though at low concentrations in the permeate streams. This created a motivation to further investigate the process by exploring the characteristics of the individual strains and their role. According to the literature reports, several reasons have been proposed for the interpretation of the passage of bacterial strains through the membranes despite their larger size than the rated pore size of the membrane [14,36-38]. One of the reasons is the possibility of the presence of defects in the membrane structure. Another possibility can be owing to the membrane aging or damage in the course of operation [39]. The biological nature of the strains can also play an important role. It has been demonstrated that microorganisms such as bacteria, cysts and parasites can exhibit flexibility in size and shape depending on the surrounding environmental conditions. This is particularly noticeable in the case of bacteria since size exclusion is not the sole mechanism for their rejection by the membranes [40]. Our findings clearly confirm the hypothesis on the effective role of bacterium size and shape. According to the trends, both membranes exhibited the largest rejection toward *Staphylococcus aureus* and *Enterococcus faecalis*, respectively, due to their spherical shape. Evidently, the larger rejection of *Staphylococcus aureus* can be attributed to its larger size compared to *Enterococcus faecalis* which retards its transport across the membrane. The three other bacteria, *Salmonella typhi*, *E. coli*, and *Pseudomonas aeruginosa*, with their coccus shape experienced fewer rejections compared to their spherical counterparts. The larger size of bacterium was corresponding to the larger rejection. Since the three bacteria are bacilli shaped, there is always the probability to pass through the membrane using their smallest width, whereas in the coccus shape bacteria, the probability of facing the membrane from every side is equal.

Mechanism of size exclusion plays an important role in MF membrane, but in the case of microorganisms the effect of the structural properties of the peptidoglycan layer of bacteria on rejection should not be ignored. For example, Lebleu et al. [41] showed on six different bacteria that the size and shape of the bacteria alone cannot be a decisive parameter for rejection. In the experiments performed on two *E. coli* and *corynebacterium* bacteria having similar size and shape, the membrane showed 3 log more rejection toward *corynebacterium* than *E. coli*. In the present study, *Staphylococcus aureus* by possessing approximately half the size of

E. coli experienced almost double rejection than *E. coli*.

The gram-negative and gram-positive bacteria are distinguished by their cell wall structure. The bacterial cell wall is composed of a specific polymeric peptidoglycan layer. In gram-negative strains, the thickness of this layer is between 2 and 6 nm, while in gram-positive strains the thickness of peptidoglycan layer is about 20 to 80 nm. Also, gram-positive bacteria have higher stiffness than gram-negative bacteria, which is attributed to the presence of peptidoglycan layer surrounding the bacteria [42]. While pores are much smaller than the size of bacteria, permission is given to gram-negative bacteria to enter the pores with a change in the structure with minimum resistance (Fig. 7).

According to Fig. 8, the polymer layer attached to the cell wall of bacteria is in charge of the mechanical strength of the cell wall. It can be assumed that the ability to deform bacteria depends on the thickness of the cell wall peptidoglycan layer. The thinner the layer, the ability to reshape and as a result the probability of passing larger bacteria through membrane pores is increased. Thus, gram-negative bacteria by having a thinner peptidoglycan layer than gram-positive experience more deformation when they pass through pores that are smaller than bacteria size. On this basis gram-negative bacteria are known as deformable particles, while gram-positive bacteria are known as stiff particles.

Obviously, the deformation and change in structure of bacteria during passing through pores can become problematic. Particularly at low pressure operations, because of less flexibility of some bacteria such as gram-positive ones, the bacteria will have intensity to be stuck in the pores or their walls and result in rapid fouling and sharp drop in the flow shortly after operation. On the other hand, due to the pressure exerting on the bacteria, the cell wall may be destroyed and lose its biological activity even after passing through membrane [38].

Two parameters can be important representatives of the bacterial structure characteristics in investigation of rejection based on size exclusion. One is the smallest cell width and another one is the cell volume. Fig. 9(a) and (b) shows the rejection results based on the smallest dimension of the bacterium cell for M-15-4 and M-17-4 membranes. The results indicate that there was no specific relationship between the smallest width of a bacterium and its rejection by the membrane. For the three gram-negative bacteria

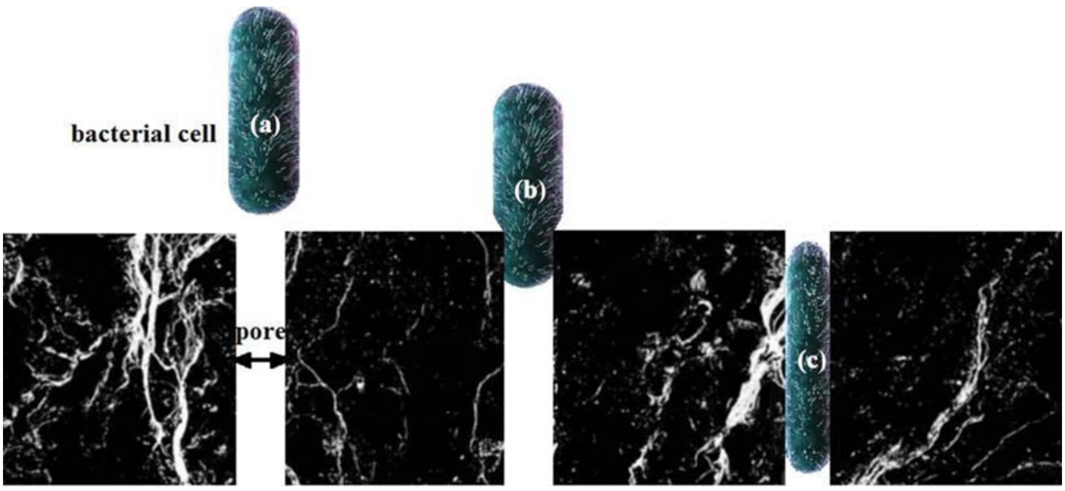

Fig. 7. The steps for the transfer of bacteria through membrane pores (a) before entering the pores, (b) deformation during entering the pores, (c) complete deformation, penetration and then passage.

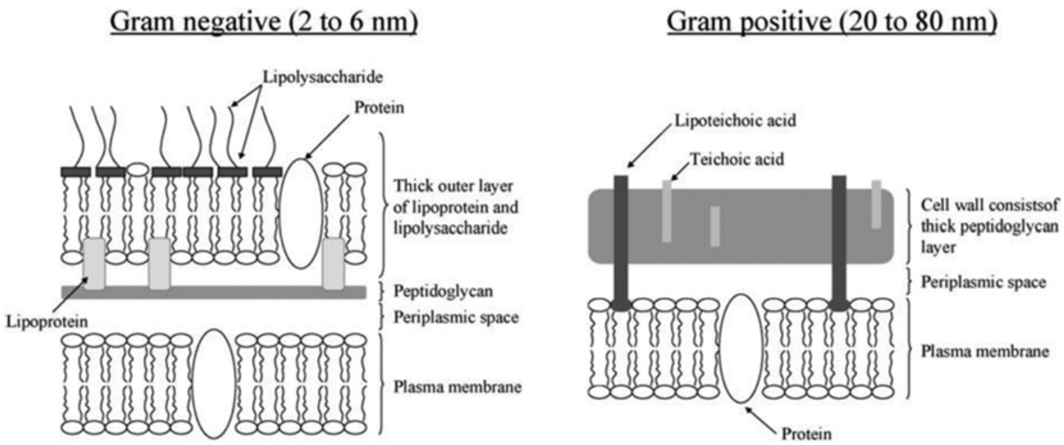

Fig. 8. Diagram of a bacterial cell wall and the peptidoglycan layer [41].

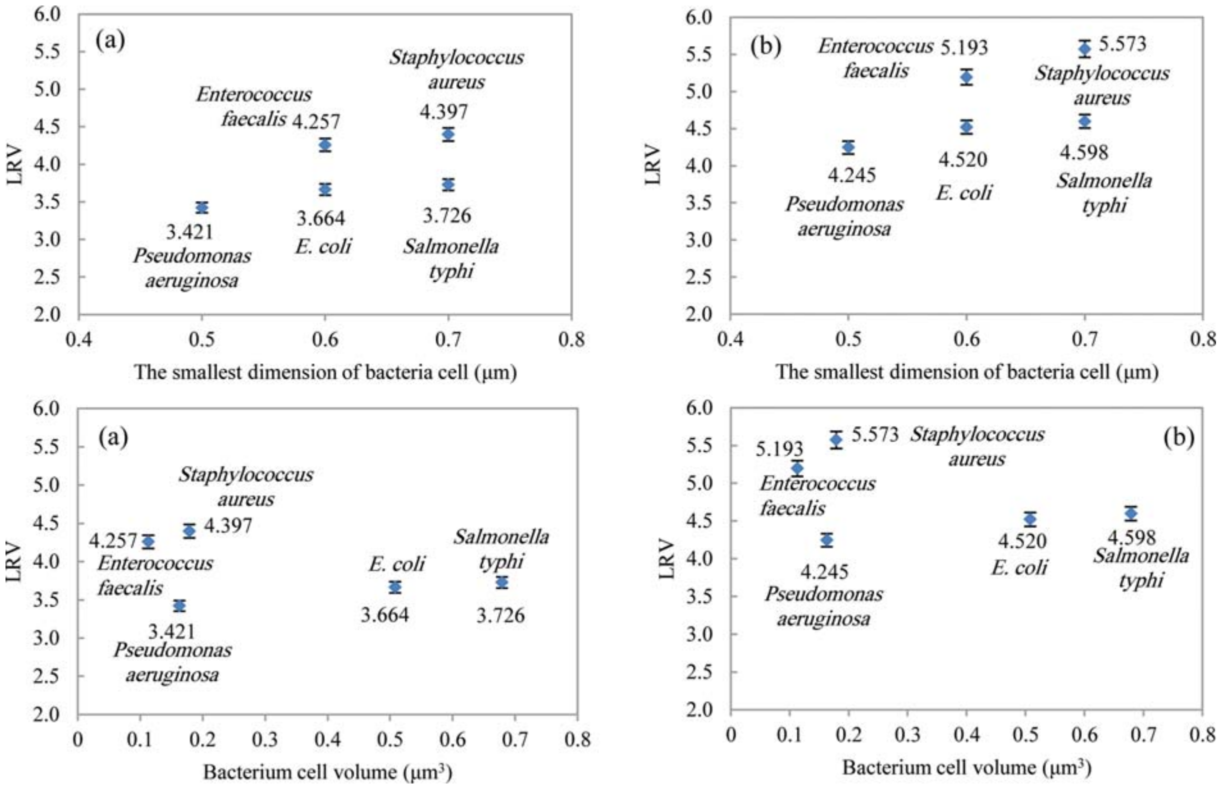

Fig. 9. The rejection results based on the smallest width of bacteria cell (upper) and bacterium cell volume (lower) for (a) M-15-4 and (b) M-17-4 membranes.

having different cell widths, almost the same rejection was seen in both M-15-4 and M-17-4 membranes. While for *Staphylococcus aureus* and *Salmonella typhi* by having the same cell width a difference between 0.7 and 1 log in rejection was seen. A similar analysis was carried out in terms of cell volume, and Fig. 9(c) and (d) show the rejection values based on the bacterium cell volume for the same membranes. The results clearly indicate that merely larger size of a bacterium than others should not be regarded as a concrete index for predicting rejection performance by a membrane [17]. Nevertheless, it may be possible to conclude that in the case of bacteria having similar strains (i.e., either gram-positive or gram-negative) or shape (coccus, rod, spirillum), the bacterium having less cell width and volume is anticipated to experience less rejection. This is in accordance with the trends observed in the case of *Staphylococcus aureus* and *Enterococcus faecalis*. According to the above interpretation, the differences in the removal efficiencies of *Staphylococcus aureus* and *Enterococcus faecalis* as gram-positive strains, compared to other gram-negative bacterial strains that had almost similar rejection, can be explained.

5. The Effect of Trans-membrane Pressure on Membrane Performance

The performance of MF membranes like other pressure driven membrane processes is largely dependent on the operating pressure. To gain an insight into the effect of operating pressure on the rejection performance of membranes, both M-15-4 and M-17-4 membranes were investigated and the results are shown in Fig. 10. It can be seen that rejection of all the bacteria was reduced upon increase in operational pressure from 1.5 to 2.5 bar. The effect was particularly significant in the case of gram-negative strains, and about 1.5 log reduction in rejection observed. However, the effect was not significant in the case of *Staphylococcus aureus* and *Enterococcus faecalis* as gram-positive strains and even a slight increase was observed in the rejection of M- 17-4 toward *Staphylococcus aureus*. Similar trends are reported by Helling et al. [43] that upon further increase in operational pressure from 2.5 to 3.5 bar, the reduction rate of rejection for gram-positive bacteria was even more on the order of 0.5-0.9 log. However, the rejection decreased by about 0.8-1.2 log in the case of gram-negative strains. It can be deduced that upon increase in the pressure, the peptidoglycan layer of the gram-positive bacteria was affected and caused deformation in the shape of gram-positive bacteria, enabling them for easier transport through the membrane at pressures beyond 2.5 bar.

Given the softer structure of gram negative bacteria compared to the gram-positive ones, they are more sensitive to deformation under pressure and this enables them for easier passage through the membrane [44,45]. Among the two gram-positive bacteria, membranes offered lower rejection toward *Enterococcus faecalis* than *Staphylococcus aureus*. This can be explained by considering the less thickness of the peptidoglycan layer of *Enterococcus faecalis* than *Staphylococcus aureus*, which makes it more flexible. Another possible reason could be the less cell width and volume of *Enterococcus faecalis* compared to *Staphylococcus aureus* (Table 1). Overall, the results confirm the large dependency of the performance of the membranes to the pressure, and membranes offer the best

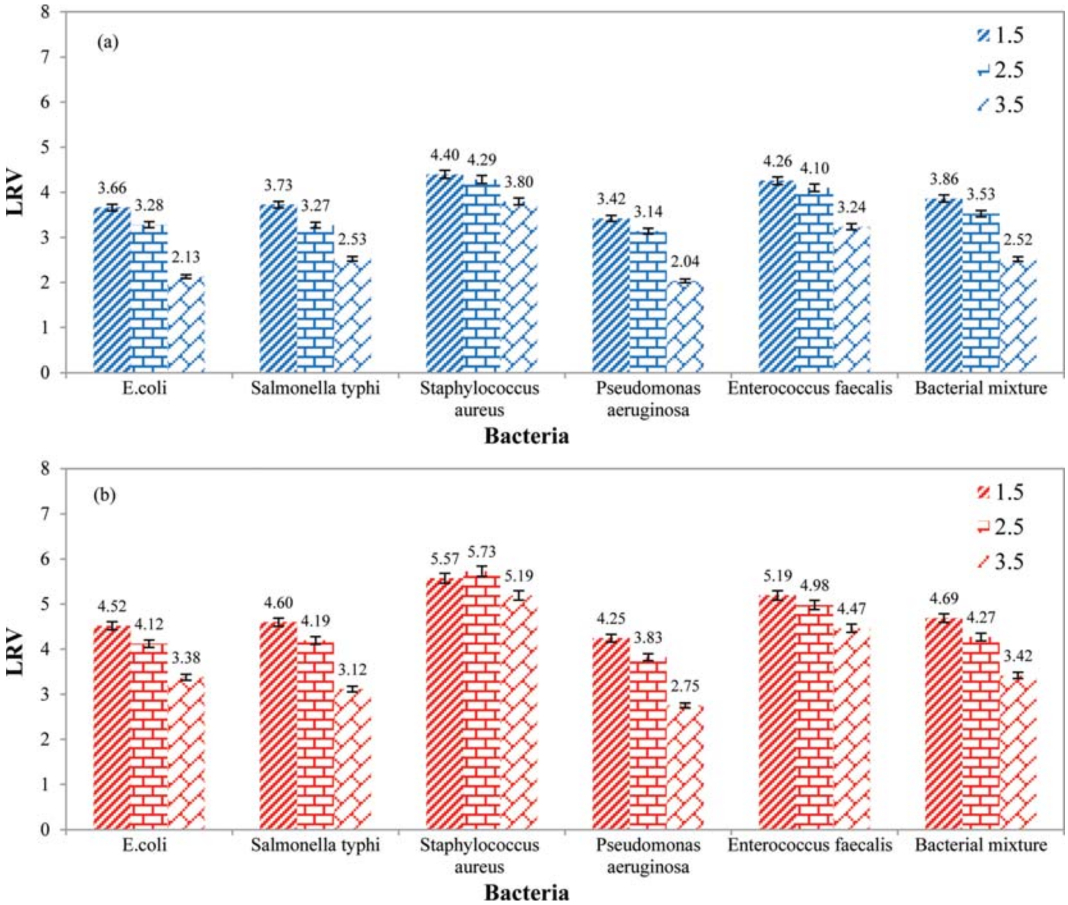

Fig. 10. The effect of trans-membrane pressure (1.5, 2.5, 3.5 bar) on rejection of (a) M-15-4 and (b) M-17-4 membranes toward various bacteria and a bacterial mixture (Operating conditions: feed conc.=10^7 cfu·ml^{-1}, pH=7.4).

rejection at the least pressure of 1.5 bar. Accordingly, investigations on the effect of other parameters on the membrane performance were continued at trans-membrane pressure of 1.5 bar.

6. The Effect of Feed Concentration on Membrane Performance

To examine the effect of bacteria concentration on the membrane performance, feeds in three different concentrations of 10^3, 10^5, 10^7 cfu·ml^{-1} were prepared using each bacteria and a mixture containing all five strains. The rejection results for M-15-4 and M-17-4 membranes, shown in Fig. 11, indicate that, in general, the rejection of membranes increased upon increase in feed concentration. Almost similar trends were observed in the case of both M-15-4 and M-17-4 membranes, though the rejection performance was better in the case of M-17-4 membranes compared to M-15-4 membranes. The observed trend can be attributed to the increased collisions of strains with each other at the entrance of pores as well as with the pore walls, resulting in the intensified pore blockage due to bridging effect [41]. Moreover, according to Gaveau et al. [42], the permeation of strains was easier at low concentrations, and aggregation phenomenon may be regarded as the responsible factor to limit the transfer of bacteria through the membranes. Thus, upon increase in the concentration of strains in feed, collisions between them especially at the pore entrances are increased. In addition, the flexibility of strains facilitates intrusion of more strains to the membrane pores. Consequently, the probability of the entrapment of the strains in the pores is increased with promoting effect on membrane rejection [46,47].

The exception in the trends was the rejection of both membranes toward *Staphylococcus aureus* and *Enterococcus faecalis* as gram-positive strains at which their rejection increased to LRV:7 at feed concentration of 10^3 cfu·ml^{-1}. The observed difference in rejection of gram-positive and gram-negative bacteria by both membranes at the least concentration can be related to their peptidoglycan layer structure [41].

7. The Effect of Feed pH on Membrane Performance

To investigate the possibility and extent of contribution of electrostatic repulsive mechanism on the performance of membranes, the effect of variation in pH on bacteria rejection was evaluated. For this purpose, *E. coli* as the representative of bacteria that are bacillus shape and gram-negative and *Staphylococcus aureus* as the representative of coccus shape and gram-positive bacteria were selected. The concentration of bacteria in feed and operating pressure was set to 10^7 cfu·ml^{-1} and 1.5 bar, respectively. According to the results in Fig. 12, upon change in pH from 5.4 to 7.4, rejection of both M-15-4 and M-17-4 membranes toward the bacteria increased. However, by increase in pH from 7.4 to 9.4, no change in the rejection of membranes was observed.

The least rejection of the membranes close to the isoelectric

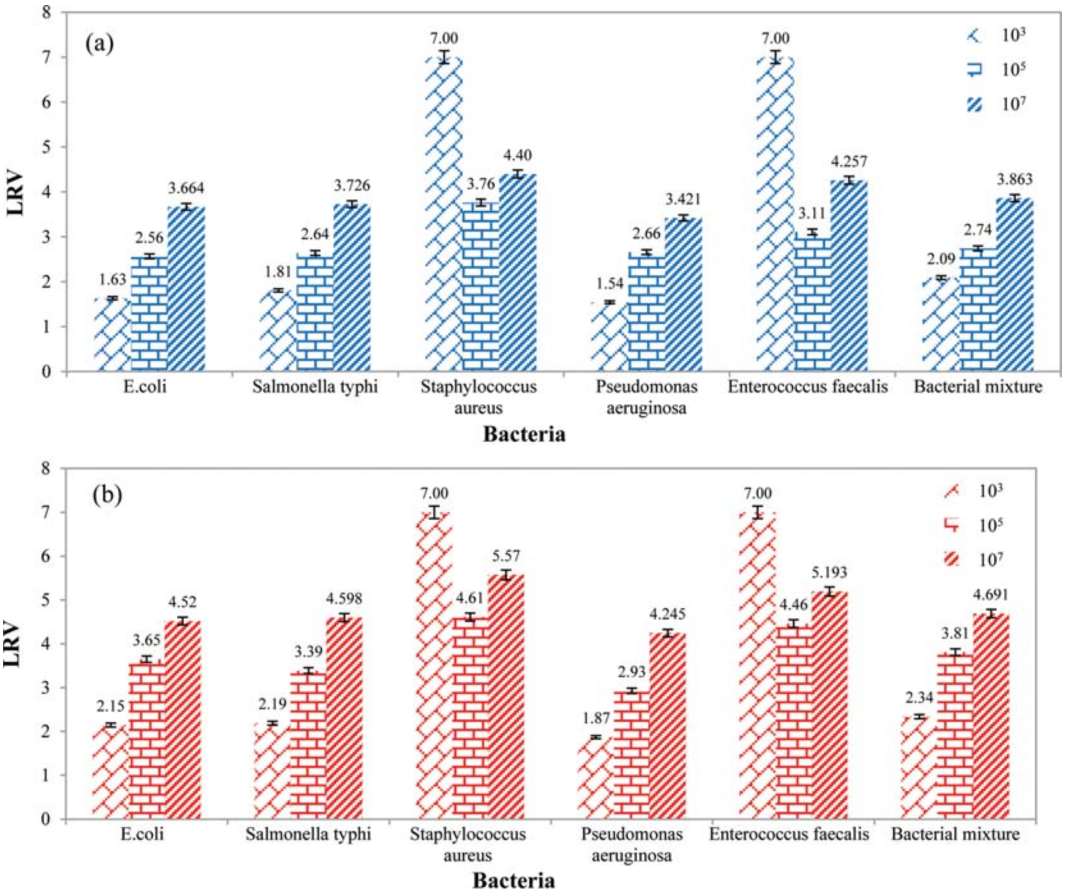

Fig. 11. The effect of bacterial concentration in feed (10^3, 10^5, 10^7 cfu·ml^{-1}) on rejection of (a) M- 15-4 and (b) M-17-4 membranes toward various bacteria and a bacterial mixture (Operating conditions: P=1.5 bar, pH=7.4).

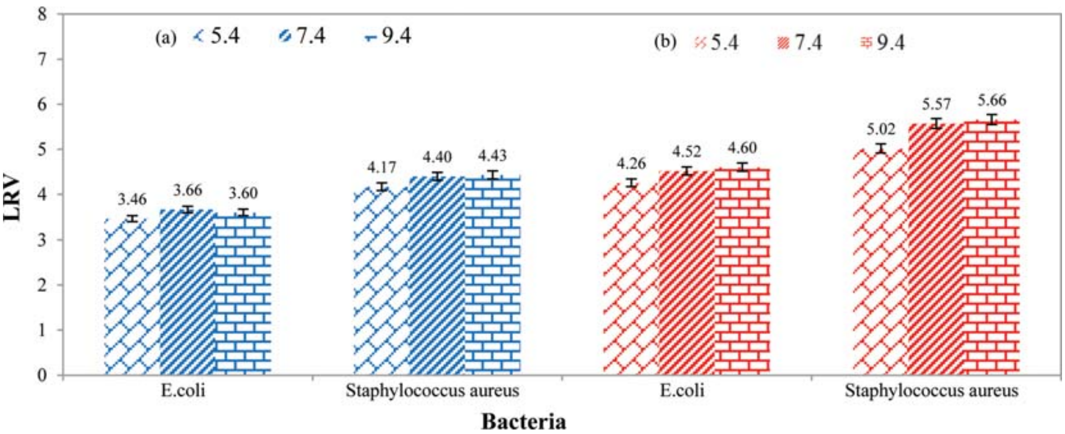

Fig. 12. The effect of feed pH (5.4, 7.4, 9.4) on rejection of (a) M-15-4 and (b) M-17-4 membranes toward *E. coli* and *Staphylococcus aureus* (Operating conditions: Feed conc.=10^7 cfu·ml^{-1}, P=1.5 bar).

point of the membrane and two bacteria (pH=5.4) suggests that the electrostatic repulsion at this point was at its least level among three different examined pHs. This is in good agreement with the findings reported by Ozaki et al. [48]. To gain more insight, the structural properties of the bacteria and membranes as well as rejection mechanisms were considered. It is well known that size exclusion plays the main role in rejection by membranes with pores smaller or comparable in size to the bacteria. However, the electrostatic repulsion mechanism becomes dominant when the pores are larger than the size of the bacteria [49,50]. Electrostatic repulsion plays its role especially when membrane and bacteria possess similar charge at their surface. The similarity at surface charge promotes the repulsive forces between membrane and bacteria which prevents the bacteria from passing through the membrane.

Table 4. The isoelectric point and zeta potential values for PAN and two bacterial strains

	Isoelectric point	Zeta potential (mv)			Ref.
		pH=5.4	pH=7.4	pH=9.4	
PAN	3.5	−40	−55	−58	[52]
E. coli	2.7	−48	−49	−50	[53,54]
Staphylococcus aureus	1.9	−35	−40	−40	[35,54]

Table 4 provides the isoelectric point and zeta potential values for membrane and examined bacteria. The isoelectric point of gram-positive bacteria varies from 1.75 to 4.15, but for gram-negative strains the variation is between 2.07 and 3.65 [51]. The larger difference in the isoelectric points of bacterial strains and membrane with the pH of the feed leads to the greater charge.

According to Table 4, the change in zeta potential for *E. coli* at pH 5.4 and 7.4 is negligible, though the rejection increased by about 0.2-0.3 log upon change in pH from 5.4 to 7.4. This may be explained considering the fact that upon increase in pH, zeta potential of the membrane increased from −40 mv to −55 mv. Essentially, increase in zeta potential is equivalent to the increase in the amount of induced charge on the membrane surface. As a result, the repulsive forces between *E. coli* and membrane surface increased with promoting effect on membrane rejection. The higher rejection of the membrane toward *Staphylococcus aureus* in comparison to *E. coli* upon variation in pH from 5.4 to 7.4 can be attributed to the increase in the zeta potential of *Staphylococcus aureus* from −35 mv to −40 mv.

It was also observed that further increase in pH from 7.4 to 9.4 did not affect the rejection of membranes. According to the data in Table 4, the zeta potential for both of the bacteria remained almost unchanged at pH greater than 7.4 though slightly increased in the case of PAN membrane. As a result, no significant change occurred in electrostatic repulsion and subsequently no effect on the rejection values.

CONCLUSIONS

The effects of various fabrication and operational parameters on the performance of PAN MF membranes for bacterial removal from drinking water were investigated. Results showed increase in polymer concentration in dope solutions from 15 wt% to 17 wt% was effective in reducing porosity and mean pore size of membranes and consequently improving membrane rejections. Although membranes prepared from higher polymer concentration showed less PWF, the rejection in the membranes reached as high as LRV:5.16 and LRV:4.87 toward *Staphylococcus aureus* and *Enterococcus faecalis*. Similarly, addition of 4 wt% citric acid to dope solutions further improved membrane rejection toward all bacterial strains. LRV rejection of the membranes containing 4 wt% citric acid towards *Staphylococcus aureus* and *Enterococcus faecalis* improved to LRV:5.57 and LRV:5.19, respectively. Overall, the best rejection results toward bacterial strains was obtained in the membrane prepared from 17 wt% PAN and containing 4 wt% citric acid in dope formulation. The results were explained by considering the particular characteristics of the individual bacterial strains including gram-positivity/negativity, shape, size and cell volume and their role in their transport through membranes. To optimize the performance of membranes, further investigations were carried out by exploring the effect of prominent operating parameters. It was found that increase in feed concentration from 10^3 to 10^5 and 10^7 cfu·ml^{-1} improved rejection performance of membranes toward all the bacterial strains as well as the bacterial mixture. This was attributed to the intensified pore blockage due to the bridging of strains as well as aggregation phenomena. The largest rejection (LRV:5.57) was observed toward *Staphylococcus aureus* at feed concentration of 10^7 cfu·ml^{-1}. On the other hand, rejection improved upon reduction in operating pressure from 3.5 to 1.5 bar. Such trend could be associated with the effect of pressure on deformation of both membrane pores and peptidoglycan layer of the bacteria. Also, shifting of feed pH to 7.4 and 9.4 enhanced membrane rejection toward *E. coli* and *Staphylococcus aureus*, to as high as LRV:4.60 and LRV:5.66, respectively. This was explained by taking into account zeta potential and isoelectric point values of involved strains and the membrane. Findings also revealed that developed PAN membranes were more effective in removal of gram-positive strains, and their rejection was strongly dependent on the peptidoglycan layer of strains. Findings in this research provide useful insights on improving the quality of drinking water through removal of bacteria by the use of MF membranes as well as setting a path forward for development of high performance polymeric membranes for use in deprived and less privileged regions around the world.

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