Preparation and performance of biofouling resistant PAN/chitosan hollow fiber membranes

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Abstract The preparation of polyacrylonitrile (PAN) hollow fiber (HF) membranes has been carried out by dry-jet wet spinning. PAN HF membranes were coated with chitosan biopolymers 2 wt% by dip coating and further crosslinked by chemical reagents (Tri sodium polyphosphate). PAN HF (Virgin) and PAN/chitosan coated membrane were characterized by SEM and tested for water flux. Proteins Pepsin, Albumin, and Clay of 1000 ppm concentration were tested for separation efficiency. In addition, bacterial species Escherichia coli and Bacillus subtilis were tested for fouling control efficiency and found out that PAN/chitosan membranes were quite superior to virgin PAN fibers. The adhesion of bacterial cells on the surface of the hollow fiber membranes assessed through alcian blue staining and SEM analysis. It was observed that PAN/chitosan membranes (310A and 310C) possessed best antibacterial activities (based on SEM results), qualifying them as a very promising candidates for anti-biofouling coatings.

Keywords Polyacrylonitrile (PAN) • Chitosan • Hollow fiber • Proteins • Biofouling

Introduction

Clean water is a serious societal issue for developing countries due to increasing in population. Nowadays, the hazardous chemicals and pathogen-free drinking water for developing countries are very difficult. Bacteria contaminated drinking water kills millions of peoples every year (Strathmann 1981; Kesting 1985; Mulder 1991; Kim et al. 2014; Prince et al. 2014; Li et al. 2014a, b). Chlorine was mainly used as a chemical disinfectant for drinking water treatment, but it creates unpleasant odor and carcinogenic byproducts. The lack of clean water is also increasing health complaints and death ratio in this world, especially in under developed countries (Monteiro and Airoldi 1999). Therefore, we need to find out the promising way for preventing the waterborne diseases, especially for under-developing countries.

Membrane technologies are widely applied in decentralized water treatment due to its low-cost, safe water obtained by single step and also it produced by different module size (Chaudhari and Murthy 2013). The main limitations of this process are membrane fouling, both organic and biofouling (Abedi et al. 2015a, b). Most of the water filtration applications have one common purpose to protect against harmful waterborne contaminants whose dimension is nanosize to microsize. Therefore, materials used for this propose should have small pore diameter with sufficient porosity (Zhang et al. 2015).

Ultrafiltration (UF) is a well-developed low-pressure membrane separation process used in different applications, such as water and wastewater treatment, reverse...
Table 1 Preparation of hollow fiber membranes

| Sample id. | 294C | 310A | 310C | 315B |
|------------|------|------|------|------|
| Dope composition (wt%) | NMP/PAN/PVP = 80/12/8 | NMP/PAN/PVP = 78/12/10 | NMP/PAN/PVP = 78/12/10 | DMF/PAN/PVP = 73/12/15 |
| BF composition (wt%) | NMP/H₂O = 50/50 | NMP/H₂O = 50/50 | H₂O = 100 | DMF/H₂O = 50/50 |
| Dope flow rate (g/min) | 2.7 | 1.8 | 1.8 | 1.6 |
| BF flow rate (g/min) | 2.0 | 1.5 | 1.5 | 1.0 |
| Dope temperature (°C) | 70 | 60 | 60 | 70 |

Fig. 1 a Schematic diagram of PAN/chitosan hollow fiber membranes. b Schematic diagram of the apparatus for permeability and rejection experiments
osmosis pretreatment and separations in the food, dairy, paper, textile, pharmaceuticals, chemicals and biochemical industries (Song et al. 2015; Low et al. 2015; Kim et al. 2014) prepared the thin-film-composite (TFC) membrane by self-assembly of the TiO$_2$ nanoparticles through coordination and H-bonding interaction with the COOH functional group of aromatic polyamide. The hybrid membrane was shown to possess the dramatic photobactericidal effect on *Escherchia coli* (*E. coli*) under UV light illumination. He observed that the 60% of *E. coli* cells in neat TFC membrane survive under dark condition without UV illumination and 37% of cells survived after 4 h UV illumination.

Fan et al. (2014) and Dasari et al. (2012) prepared the electrospun polylactic acid (PLA) membranes containing functionalized sepiolite fibrillar particles (5 wt%). Biocidal activity was achieved by functionalizing sepiolite fibers with Ag (26 wt%) and Cu (26 wt%), which were embedded in the fiber surface. He achieved that the effect was particularly intense for Ag-sepiolite in contact with *Saccharomyces cerevisiae*, where the reduction amounted to 85% compared to neat PLA (control) (Mondal and De 2016) and Sawada et al.) prepared acrylamide grafted onto a polyethersulfone (PES) hollow fiber membrane and silver nanoparticles were then formed within the acrylamide layer. Antibacterial activity of the silver-loaded membrane was evaluated against *Escherichia coli* (*E. coli*) using the halo zone test with an agar culture medium. He achieved that 99.999% of *E. coli* cells were killed, suggesting that the silver nanoparticles were quite useful for inhibiting bacterial growth (Mu et al. 2012; Dutta et al. 2004; Li et al. 2014a, b).

Hollow fiber ultrafiltration (UF) membrane is the most mature and advanced UF technology used in food, pharmaceutical, chemical, and water treatment industry (Cabasso et al. 1976, 1977; Gao et al. 2016). Because the hollow fiber UF membrane has high spatial use factor and large surface area, it has good separation capability (Prince et al. 2014; Guo et al. 2010; Celik et al. 2011; Xu et al. 1999).

Polyacrylonitrile (PAN) is one of the versatile polymers, which is widely used for fabricating membranes due to its good solvent resistance property (Moafi et al. 2011). PAN-based ultrafiltration (UF) membranes are widely applied in various industrial applications due to its thermal stability, resistance to most of the organic solvents, bacteria, and photoinitiation (Demir 2002; Tasselli et al. 2004; Tasselli et al. 2005; Aptel et al. 1985; Tasselli and Drioli 2007). However, these membranes are severely affected by membrane fouling due to its poor hydrophilicity and

![Fig. 2 SEM images of the 294 C virgin and coated hollow fiber membranes (A1, B1 external surface; A2, B2 cross-sectional images)](image-url)
biocompatibility. It decreases the water flux and increase the filtration cost (Li et al. 2014a, b; Booshehri et al. 2013; Ahsan et al. 2015; Saufi and Ismail 2002; Ren and McCutcheon 2015; Oh et al. 2001).

To improve the antifouling property of PAN-based hollow fiber membrane, surface modification techniques were carried out, i.e., plasma treatment, coating, surface graft polymerization, and chemical treatments. Recently, coating of biopolymers is one of the promising ways to produce low-cost antifouling membrane (Childress et al. 2005; Germic et al. 1997; Liuxue et al. 2006; Prama Ekaputra et al. 2015; Acharyulu et al. 2013; Marelli et al. 2010; Feng et al. 2015; Han and Nam 2002; Mei et al. 2012). Chitosan was one of the promising cationic biopolymers with wide applications due to its biocompatibility, biodegradability, non-toxicity, chemical and thermal stability, capability to form gels and films, etc. (Yoon et al. 2006; Chaudhari and Murthy 2013; Abedi et al. 2015a, b; Wang et al. 2007; Tao et al. 2012).

PAN fibers produced under various casting conditions (Table 1) codes as 294C, 310A, 310C, and 315B were shortlisted for this investigation. The surfaces of these PAN fibers were modified by coating of chitosan biopolymer. The resulting PAN-Chitosan membranes were characterized by scanning electron microscope (SEM). The separation efficiency of the virgin and biopolymer coated fibers is tested for proteins, albumin, pepsin, and clay (kaolin light). Antifouling of PAN nanofibrous membrane containing both hydrophilic spacer and antibacterial agent was already developed by (Morelli et al. 2010). However, these nanofibrous membranes do have antibacterial and antifouling properties, but to enhance the capacity, chitosan seems to be very promising agent. The antibacterial properties of PAN hollow fibers were evaluated by Gram-positive bacterium Bacillus subtilis and Gram-negative E. coli.

Materials and methods

Materials

The membrane material PAN was a copolymer containing 92% of acrylonitrile and 8% of vinyl acetate, with a viscosity average molecular weight of 40,000 Da, supplied by MontefibreSpA, Italy. Polyvinylpyrrolidone (PVP K17), supplied by BASF, Germany, was used as pore-former and N-Methyl-2-pyrrolidone (NMP) and N,N-Dimethylformamide (DMF) (Sigma-Aldrich) as the solvents. Chitosan (Viscosity 50–100 mPa.s, TCI make) as coating material.

Fig. 3 SEM images of the 310A virgin and coated hollow fiber membranes (a1, b1 external surface; a2, b2 cross-sectional images)
Preparation of PAN HF membranes

The preparation of PAN HF membranes has been carried out according to the dry-jet wet spinning (or simply dry–wet spinning), the well-known process for the preparation of hollow fiber membranes. According to this process, the polymer solution is pumped through a spinneret, and after a short residence time in air (dry phase), the nascent fiber enters a coagulation bath (wet phase) where it takes places the formation of the membrane (Tasselli et al. 2005). The polymer solution (dope) was prepared by mixing under mechanical stirring the polymers and solvent at 60°C and kept at the same temperature during spinning test. The dope and the bore fluid (BF) composition along with the most representative operating conditions were reported in Table 1.

The prepared HFs, collected as a continuous bundle, were cut into pieces of about 30 cm and washed in water for two days, refreshing several times with water. Then, the HFs were kept for 24 h in 20% glycerol solution (to prevent the pore collapse upon drying) and finally dried at ambient conditions (Oh et al. 2001; Wang et al. 2007; Pierog et al. 2009).

Coating procedure for PAN hollow fiber membranes

1000 mg/L chitosan solution was prepared by dissolving 1000 mg of chitosan in 1 L distilled water. Acetic acid was added in distilled water to reduce its pH to 2.5 prior to chitosan addition. Acetic acid was required to “protonize”—NH₂ groups of the chitosan and make it soluble in water. Trisodium polyphosphate of suitable concentration was added into the solution for crosslinking purpose. The solution was stirred with magnetic stirrer at 500 rpm at 50°C. PAN hollow fiber membranes coded as 294C, 310A, 310C, and 315B were preheated at 25°C in an oven for about 15 min for surface activation and then dipped into chitosan solution. This is left undisturbed for 12 h in fume-hood. Then, the fibers are taken out and dried in oven for about 15 min at 50°C. The dried PAN/Chitosan hollow fiber membranes were stored in an air-tight pack.

Preparation of PAN/Chitosan hollow fiber modules

The experimental modules were made in glass, with the size of 18 cm (Length) × 2.5 cm (Dia). The top and bottom of the glass tubes consist of holes for the insertion of

![Fig. 4](image-url)  
*Fig. 4* SEM images of the 315B virgin and coated hollow fiber membranes (a1, b1 external surface; a2, b2 cross-sectional images)
fibers. The inlet and outlet were placed near top end and bottom end in opposite direction. The coated fiber are inserted through top holes and taken out by the other end and holes were sealed by epoxy resin to make a leak-proof seal, which is hydrophobic with thermo-setting capacity.

Preparation of protein solution for rejection studies

The 1000 mg/L of various proteins such as albumin, pepsin, and clay were prepared with distilled water. Prepared solutions were allowed to run for about 15 min. Permeate was collected by outside-in filtration through hollow fibers in recirculation mode. The sample collection was repeated for three times to ensure the reproducibility.

The percentage rejection was calculated by the following equation:

\[
\% \text{ Rejection} \; R (%) = \frac{C_f - C_p}{C_f} \times 100
\]

where Cf and Cp are the feed and the permeate concentration, respectively.

Experimental setup

The schematic diagram of PAN/Chitosan hollow fiber membranes was shown in Fig. 1a and experimental setup was shown in Fig. 1b. It was clearly explained that one end of the inlet PVC pipe will be inserted in a conical flask. The other end of the pipe will be inserted to the ultrafiltration motor. From the outlet of the motor, the pipe will be connected to the inlet of the module. The pipe at the outlet end of the module will be connected with a pressure gauge. The pipe from the pressure gauge will be inserted into the conical flask to complete the circle. The module was kept inside a large petri-plate to collect permeate.

Membrane morphology

The hollow fiber membrane (PAN and chitosan coated PAN/CHI fiber) morphology (cross-section and surface) was examined using a scanning electron microscope FESEM JSM-7100F. The samples of the composite fiber were frozen and fractured in liquid nitrogen and subsequently gold sputter coated for analysis.

Bacteria culture preparation and biofilm adhesion test on membrane

The test bacteria (E. coli and B. subtilis) were cultivated in 30 ml of culture tube with LB (Luria–Bertani) and ZMB (Zobell Marine Broth) broth purchased from Himedia.
(India) at 30 °C with shaking at 180 rpm. The bacteria culture was harvested in their exponential growth phase using centrifuge (Sigma, 3K30, UK) at 5000 rpm for 10 min at 4 °C. To test the bacterial adhesion on the surfaces of the membranes, the shake flask method was carried out. All membranes were sterilized using ultraviolet radiation in laminar air cabinet (Esco, USA); four-to-five pieces of 1 cm size of hollow fiber membrane were placed in all the bacterial suspension at 30 °C with shaking speed 180 rpm for 24 h. In the bacterial biofilm growth on the membranes, studies carried out by sterile zones microscope (Olympus, SZX16) and SEM (JEOL, JSM7100F) (Kim et al. 2014). The bacterial biofilm formation on the surface of membranes was confirmed by alcian blue staining. The membrane in the incubation medium removed after 24 h contact time, which was washed with mili-Q water to remove the merely adhered bacteria on the surface. Membranes were tested for the presence of biofilm formation on the surface. The membranes were incubated in 0.5% of the alcian blue solution for about 30 min, after that it was removed and washed with distilled water to remove excess stains. The blue color development identified using stereo zoom microscope. For observation of bacterial biofilm on the surface of hollow fiber membrane, scanning electron microscopy was performed by fixing membrane in 3% glutaraldehyde for 10 min. post-fixation with 1% osmium tetroxide was carried out for 30 min. Dehydration was performed with increasing concentration of absolute ethanol, 50, 80, 95, and 100%. Sampled were dried and coated with gold prior to imaging with SEM (Jaiswar et al. 2017).

Results and discussions

SEM of the prepared PAN/chitosan ultrafiltration hollow fibers

The morphology and size of the virgin hollow fibers is mainly due to the different operating conditions under which they were prepared. Different solvents, dope composition and flow rate, bore fluid (BF) composition, and flow rate, yield different hollow fiber size and morphology. Although the discussion of the influence of such parameters on the membrane morphology is beyond the scope of this work, some general considerations can be done:

- The higher the dope flow rate and the BF flow rate, the higher the dimensions of the hollow fibers.
- The coating procedure generally makes more even with lower pore size the outer surface of the hollow fibers.

In particular, the membrane outer surface and cross-sectional view of 294C virgin and chitosan coated hollow fiber membrane were shown in Fig. 2. Virgin PAN surface has an average pore size of 0.77 μm with uneven rough surface which allows more permeation flux. The coated
Fig. 7 PAN/Chi 310A fiber with a *Bacillus subtilis* and b *E. coli* after alcian blue staining (24 h incubation)

Fig. 8 SEM images of *Bacillus subtilis* on 310A hollow fibre membrane after 24 h of incubation (a virgin; b coated)
membrane surface has smaller pores with even surface which determines a lower permeation flux and a much higher rejection of large size particles such as proteins, clay, or bacteria (Kapantaidakis et al. 2002; Drioli and Haichun 1989). The outer diameter of virgin membrane (415 μm) and chitosan coated (507 μm) indicates the presence of additional surface Chitosan layer as well as swelling of PAN fibers. From the results, it was observed that the coated membrane pore size reduced compared to virgin PAN fibers.

The cross-sectional view of the 310A uncoated fiber has thick outer layer with single finger-like layer and uniform porous structure which helps the perfect separation of proteins and pathogens (Fig. 3). This may be due to the different microscopic structure which, for some reason, seems more open in the coated fibers. Virgin 315B hollow fiber (Fig. 4) shows a less dense surface morphology than chitosan coated 315B hollow fiber, which is a hurdle for the filtration process.

The membrane top surface and cross-sectional view of 310C virgin and chitosan coated hollow fiber membrane were shown in Fig. 5. The surface of virgin membrane is uneven and has shell-shaped structure. However, the coated fiber surface has even pores which are smaller compared to 310A fiber. The cross-sectional images also prove this.

**Rejection analysis**

**Protein adsorption studies**

The rejection experiments were carried out with pepsin, albumin, bacteria, and concentrations which were measured before and after rejection by ultraviolet spectrometer at suitable wavelength.

The rejection percentage for clay was analyzed by turbidity meter and percentage rejection calculated by the following formula:

\[
\% \text{ Rejection} = \frac{\text{final OD} - \text{initial OD}}{\text{initial OD}} \times 100.
\]

**Protein rejections of hollow fiber membranes**

The protein rejection studies of virgin and coated membranes were shown in Fig. 6. From results, it was observed that chitosan coated fibers showed better removal or similar removal percentage but provide additional fouling control property. Clay removals by chitosan coated fibers are above 90% and match with virgin PAN fibers. Albumin rejection using 294C, 310A, 310C membranes is 76–85% compared to less rejection of pepsin (50%). Out of all fibers, 310A and 310C performance (protein and clay removal) was found to

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**Fig. 9** SEM images of *E. coli* on 310A hollow fibre membrane after 24 h of incubation (a virgin; b coated)
be best and 315B shows poor performance. Protein rejection values mainly depend on membrane morphology (phase-inversion mechanism). In addition to the membrane morphology, the size-sieving mechanism is more pronounced in chitosan coated membranes due to smaller pore size. The strong interactions between polar groups of chitosan (amino and hydroxyl) and functional groups of proteins and clay may determine the separation. The membrane surface has active amino groups and residual amide groups in its backbone. Overall synergistic effects are key behind rejection of the coated membranes. Therefore, apart from morphology (determines membrane performance), chemical interactions is also a deciding factor.

In addition to that coating a biopolymer, chitosan on membrane surface which we are investigating the biofilm control gives direct indication that there was no protein deposit on membrane surface. By reducing protein deposits reduces the fouling and improve separation properties of the membrane.

**Observation of bacterial biofilm formation through stereo zoom and SEM**

The attachments of bacterial cells on hollow fiber membranes surface assessed through alcian blue staining and SEM study. Out of the four membranes studied, No. 310A and 310C show very prominent antibacterial activities based on SEM (Figs. 8, 9) analysis. It has been observed during alcian blue staining that, virgin PAN membrane shows blue color precipitate on its surface (Figs. 7, 10) indicating the bacteria growth. Whereas chitosan coated membrane does not show blue color indicating non adherence of bacterial cells, since the alcian blue staining is qualitative test. Further SEM study confirms the antibacterial property of the membranes.

Bacteria which covered the virgin PAN hollow fibrous membrane surface indicate the inability to kill bacteria, whereas CHI coated fiber prevents bacteria growth (Fig. 10). 310C membrane is reported as best membrane in terms of microorganism removal. Cells are clearly visible after 24 h of incubation (Figs. 11, 12) of virgin membranes, although cellular disruption started confirming the antimicrobial property under prolonged period as reported earlier by Mukherjee et al. (2017). It was found that Chitosan coated PAN membranes (310A and 310C) possessed high antibacterial activities. The number of cells reduced to 0.013 and 0.014 cells/ml from 1.126 cells/ml (initial count) for *B. subtilis* and 0.002 and 0.187 cells/ml with the initial concentration of 1.294 and 1.329 cells/ml in the case of *E. coli*. This better antibacterial efficiency might be
Fig. 11 SEM images of *Bacillus subtilis* on 310C hollow fibre membrane after 24 h of incubation (a virgin; b coated)

Fig. 12 SEM images of *E. coli* on 310C hollow fiber membrane after 24 h of incubation (a virgin; b coated)
obtained by the amides in the enlarged surface area of the hollow fiber membranes (Fig. 12).

Conclusion

Virgin PAN membranes have cracked surface, whereas chitosan coating provides denser morphology. Biofouling comprises of a quite complicated reaction pathway and sources of biofouling. Here, the possible mechanism may be due to the chitosan coating which has active amide group on its backbone responsible for controlling the bacterial deposits on membrane surface. The overall investigation shows that 310C and 310A fibers were suitable for separation process than other fibers due to membrane morphology. The adhesion of bacterial cells on the surface of the hollow fiber membranes assessed through alcian blue staining further visualized by SEM analysis. It was found that Chitosan coated PAN membranes (310A and 310C) possessed high antibacterial activities, which qualifies them as a very promising candidates for anti-biofouling coatings. Our research group is planning to extrapolate this work especially on mechanism pathway using more biological analysis in future.

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Compliance with ethical standards

Conflict of interest All authors declare that they have no competing interest.

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