

Synthesis and characterization of ultrafiltration membranes by phase inversion and by uropathogenic *Escherichia coli* retention performance

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ABSTRACT

Ultrafiltration (UF) membrane was prepared using the Manjikian process via phase inversion from casting five increasing polymer concentrations (cellulose acetate at 15, 16.5, 18, 19.5 and 21 wt.%), acetone and formamide (2:1) (v/v). The synthesized membranes were tested by Fourier transformed infrared spectroscopy, scanning electron microscopy, contact angle and porosity. The permeability was determined and the molecular weight cut-off was evaluated by the retention of polyethylene glycol. The results showed that the higher polymer concentration enhanced hydrophilicity of the membranes as well as decreased the molecular weight cut-off. The membranes demonstrated greater potential to retain pathogenic and epidemiological UroPathogenic *Escherichia coli* (UPEC) at higher polymer concentrations. The highest polymer concentration membranes were able to retain a total discharge of 8U-log bacteria.

Keywords: Ultrafiltration membrane; Phase inversion; Cellulose acetate; PEG; UPEC; Epidemiology discharge

1. Introduction

Membrane separation processes have recently attracted considerable interest for their potential applications in sterile filtration of pharmaceuticals, water desalination, textile and production as well as removal of micro-pollutants [1,2]. Membrane filtration is effective to separate and concentrate small molecules with an efficient and scalable methodology utilizing few or no chemicals with no harmful by-product formation. Membranes can be synthesized through a variety of processes to retain a range of particle size dependent on membrane pore sizes [3]. Membrane separation processes include microfiltration (MF), ultrafiltration (UF), reverse

osmosis (RO), and electro-dialysis (ED); these processes are used in many industries for the recycling of rare metals, toxic chemicals, biomolecules, polymer binders, and colloidal particles [4]. Among the membrane processes available, UF represents the most commonly used method to separate desirable and undesirable components present in a given solution. According to literature, the ultrafiltration has several applications as an example; the production of drinking water [5,6]. In addition, Devereux et al. [7] examined the scale-up of hollow-fiber UF for the concentration of protein. Further, the UF has been proven to eliminate high organic load [8]. In fact an enzymatic process of cellulose hydrolysis based mainly on the use of membrane techniques was

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studied by Pizzichin et al. [9]. First synthetic membrane was created in 1963 by a phase inversion process using cellulose acetate (CA) by Loeb and Sourirajan [4]. The phase inversion method is used for asymmetric membrane synthesis [10,11]. In this method, the solution film is immersed in a water bath for flow precipitation. During the process, the solvent in the casting solution film is exchanged with non-solvent and phase separation occurs on the film. The process produces the characteristic morphology of asymmetric membrane with a dense top layer and porous sub-layer [12]. The polymer CA is often selected for cost-effectiveness and chlorine resistance. In addition CA is hydrophilic, resists fouling and acceptable biocompatibility as a sustainable resource [13–15].

Urinary tract infections (UTIs) are among the most common community-acquired and nosocomial infections [16,17]. UTIs are often acquired by people already ill or in the hospital and are rarely fatal. However, UTIs are treated with antibiotics, and thus contribute to the increasing risk from antibiotic resistant microbes. Uropathogenic *Escherichia coli* (UPEC) strains are the most common bacteria implicated in UTIs. In recent decades, multidrug-resistant UPEC have led to major challenges in treating infected patients [18] and recent studies have investigated UPEC virulence [19,20]. Conventional wastewater treatment is generally thought to reduce the numbers of enteric bacteria. Waterborne pathogens and related diseases are a major public health concern worldwide, not only from morbidity and mortality but also from high costs in prevention and treatment.

Waterborne pathogens are directly linked to environmental deterioration and water pollution [21,22]. To minimize the risk of fecal bacteria pollution to watersheds (including pathogenic *E. coli*) effluents generally are disinfected using oxidative processes to destroy or deactivate these organisms. Chlorine is the most commonly used disinfectant [23]. Alternative processes such as ozonation and UV irradiation are currently used for disinfection in many countries. However, chlorine generates several by-products harmful for both human and the environment [24,25]. UV germicide lighting also has limited success in disinfecting water as some bacterial strains may become more virulent in the process [26,27]. The use of membranes for bacteria removal from water is promising in both the removal of all potential pathogens with no chemical by-products. In addition, defining the pore size of a synthesized membrane may reduce the risk of clogging during the filtration process [28,29]. Shang et al. [30] reported outstanding removal of *E. coli* by a membrane bio-reactor.

In this context, the objective of this study is (i) to synthesize a range of UF membranes from casting solutions varying from 15 to 21 wt% of CA, (ii) to characterize the synthesized membranes by pure water permeability, scanning electron microscopy (SEM), Fourier transformed infrared spectroscopy (FTIR), and (iii) to apply the retention of one epidemiological strain of UPEC.

2. Material and methods

2.1. Material

Cellulose acetate with an average molecule weight of 30,000 g/mol (39.8 wt.% content, degree of substitution 2.5)

(CA) was used as the membrane forming polymer (Fig. 1). Acetone (NMP > 99%) was used as the solvent for CA while formamide is not a solvent, polyethylene glycol (PEG1000 PEG4000, PEG6000, PEG8000 and PEG20000) were purchased from Sigma-Aldrich (Sfax-Tunis).

2.2. Membrane preparation

A series of CA UFs were prepared by phase inversion method in water as a coagulant bath. The polymer CA membranes were cast by knife on a glass plate from 15–21 wt.% polymer solution in mixture of acetone and formamide (2:1) (v/v). The dope solution was poured onto a glass plate, and was spread with a doctor's blade to a 200 μm thickness. The glass plate was immediately immersed in a distilled water coagulation bath (fresh distilled water with neutral pH at ambient temperature) at 4°C without evaporation time for 1 h. The membranes were then annealed for 10 min in a distilled water bath at 60°C [14]. The nomenclatures of membranes were MR- x - y where x denoted the annealing temperature and y number of membrane. The composition of the UFs synthesized are summarized in Table 1.

2.3. Membrane characterization

Quantitative analysis of CA membrane was performed by Fourier transformed infrared spectroscopy (FTIR). The identification was carried out in the range 0–4,500 cm^{-1} using a FTIR IR affinity⁻¹ (Shimadzu, Kyoto, Préfecture de Kyoto, Japan) [31].

The images of the membranes were taken with a scanning electron microscope JEOL (Japan Electro Optic Laboratory, model JSM 5400A, Peabody, Massachusetts, USA). The membranes were freeze-fractured in liquid nitrogen to give a generally consistent and clean break and were then sputter coated with a thin film of gold. The images were made at 15 kV with a magnification value (or factor) ranging between 200 and 2,000.

The contact angle was measured by the water-drop method on an optical camera using a tensiometer of Attension Theta T200 brand. De-ionized water was carefully dropped on the top surface and the contact angle between the water and membrane was measured until no further change was observed. In all measurements, to minimize the experimental error, the contact angle was measured at five random locations for each sample and then the average value was reported.

Pure water flux (PWF) of the different membranes was performed after membrane compaction at a trans-membrane pressure of 6 bar for 4 h. The PWF was determined using

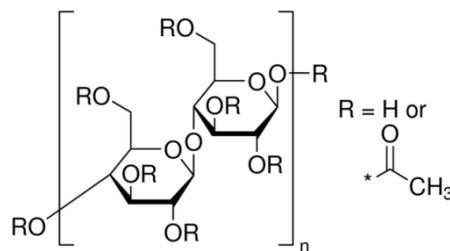


Fig. 1. Chemical structures of cellulose acetate.

Table 1
Composition of the CA UF membranes synthesized

Membrane name	Solution composition (wt.%)		
	Polymer	Acetone	Formamide
UF-60-1	15	56.66	28.33
UF-60-2	16.5	55.66	27.83
UF-60-3	18	54.66	27.33
UF-60-4	19.5	53.66	26.83
UF-60-5	21	52.66	26.33

stainless steel cell (Millipore, Burlington, Massachusetts, États-Unis) having a total volume of 100 mL. The effective membrane area is of 15.54 cm². The ultrafiltration set-up is reported in Fig. 2.

The PWF was measured according to the Darcy's law (Eq. (1)):

$$L_p = \frac{J_v}{\Delta P} \quad (1)$$

With

$$J_v = \frac{Q}{A\Delta t} \quad (2)$$

where L_p is the water permeability (L h⁻¹ m⁻² bar⁻¹), ΔP is the pressure, J_v is the PWF (L m⁻² h⁻¹), Q is the quantity of permeate (L), A is the effective membrane area (m²) and Δt is the sampling time (h).

The molecular weight cut-off (MWCO) value was determined by using PEG series of increasing molecular weights. Membrane PEG rejection coefficient is determined by Eq. (3).

$$\%R = \left(1 - \frac{C_p}{C_f}\right) \times 100 \quad (3)$$

where C_p and C_f (mol/L) are the concentrations of the permeate and feed solutions, respectively.

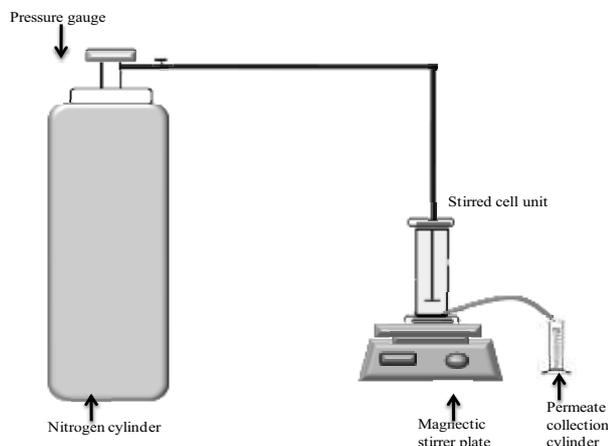


Fig. 2. Ultrafiltration set-up.

The MWCO was determined using PEG with different molecular weights (PEG1000, PEG4000, PEG6000, PEG8000 and PEG20000). It is defined as the lowest molecular weight (in Daltons) at which greater than 90% of a solute with a known molecular weight is retained by the membrane [32]. All the PEG solution ($C = 10^{-3}$ mol L⁻¹) were prepared at pH = 7.3, ambient temperature and the trans-membrane was fixed at 2 bar.

The water content of the membranes was obtained after soaking membranes in water for 24 h; the membranes were weighed followed by mopping them with blotting paper. After filtration, the permeate solution was determined for PEG concentration using total organic carbon analyzer (Sievers Innovox ES).

The water content of the membranes was obtained after soaking membranes in water for 24 h; the membranes were weighed followed by blotting with blotting paper. The wet membranes were placed in vacuum drier at 75°C for 48 h and the dry weights of the membranes were determined [33]. The percentage water content was calculated by the following equation:

$$\% \text{ Water content} = \frac{\text{Wet sample weight} - \text{dry sample weight}}{\text{Wet sample weight}} \times 100 \quad (4)$$

The average pore size, pore size distribution and bubble point of the membranes were determined using a PMI Capillary Flow Porometer (CFP1500 AEXL, Porous Materials Inc., USA), the membrane was wetted using Fluorinert FC-40 for 24 h then was placed in the membrane support. Bubble point, gas pressure and flow rates through the dry membranes were determined. The operating mode, named wet-up/dry-up, was selected using the software CapWIN. The measurement of average pore size, pore size distribution and bubble point was determined using the Laplace's equation (Eq. (5)):

$$dp = \frac{4\tau\cos\theta}{p} \quad (5)$$

where dp is the pore diameter, τ is the surface tension of the liquid, θ is the contact angle of the liquid and p is the external pressure. The results were exported as an excel file using software Caprep.

2.4. Removal of UPEC from water using the synthesized membranes

To estimate the performances of each synthesized membrane (UF-60-1; UF-60-3 and UF-60-5), overnight cultures of UPEC strains in nutrient broth were transferred and mixed with distilled sterilized water to make a final volume of 1 L. The bacterial load was determined by serial dilution before and after membrane treatment. Using plate count agar, the viability of cells was checked.

3. Results and discussion

3.1. Fourier transform infrared spectroscopy

The FTIR analysis was utilized to study the chemical characterization of the synthesized CA membranes. The FTIR

spectra of clean CA membranes are shown in Fig. 3. The FTIR spectra shows the bands of the cellular skeleton, with a broad band between 3,600 and 3,300 cm^{-1} attributed to the non-acetylated OH bonds of the cellulose [34]. The absorption band values seen at 2,950 cm^{-1} are attributed to the asymmetry stretching vibration of CH_2 [35]. The strong peak at 1,754 cm^{-1} corresponds to stretching vibration of the group $>\text{C}=\text{O}$ [36] followed by peaks at 1,370, 1,220 and 1,060 cm^{-1} that capture C–O stretching of ester, C–O stretching of carboxylic acid, and C–O stretching of ether, respectively [35].

3.2. Scanning electron microscope

The SEM was widely used for its ability to analyze the morphology, the cross section, top surface and bottom of the membranes. The SEM images of the prepared membranes are presented in Fig. 4. It is clear that all the membranes have an asymmetrical structure consisting of a dense top layer and a porous sub layer that was occupied by cellular morphology enclosed in the polymer matrix, as well as finger-like macrovoids. According to these images are randomly distributed on the membrane surface. Indeed when the casting solution was immersed in water coagulation bath the macromolecules of CA at interface aggregated rapidly so that a dense skin was formed while the evaporation of solvent lead to the formation of macrovoids. The increase of CA in the casting solution controls the morphology of asymmetric membranes and the water permeability [37].

3.3. Pure water permeability

The permeability of water is obtained by measuring the flow at different pressures and by applying the Darcy's law [Eq. (1)]. The influence of polymer concentration on pure water permeability (PWP) of CA UF was investigated, in order to find the possible improvement in the efficacy of the membranes, which is represented in Fig. 5. From the result we can note that the water permeability of all the membranes increased with increasing applied pressures since the driving force for permeation of water was enhanced. This fact is clearly demonstrated from the values obtained for UF-60-1 and UF-60-5. The permeabilities were 36.83, 29.82, 25.6, 19.95 and 15.98 $\text{L m}^{-2} \text{h}^{-1} \text{bar}^{-1}$ for UF-60-1, UF-60-2, UF-60-3, UF-60-4 and UF-60-5, respectively. The pure water

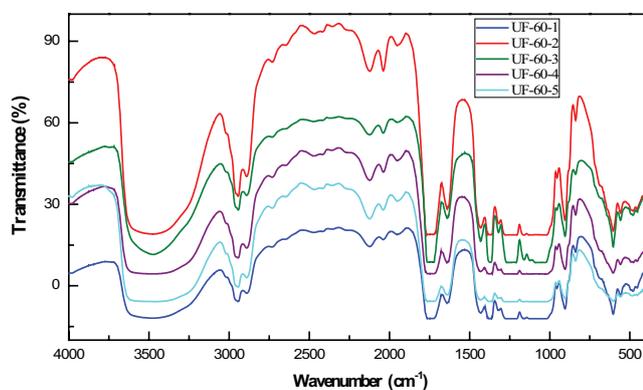


Fig. 3. Determination of the FTIR spectra for the different membranes.

permeability depends on the polymer concentration. It is seen that the pure decreasing upon increase in the concentration of the polymer CA. The increase in polymer concentration decreased the PWF because greater number of pores formed.

3.4. Determination of the molecular weight cut-off

The determination of MWCO of the synthesized is represented in Fig. 6. The role of different concentrations CA in bland of membrane was investigated. They show that UF60-1, UF60-2, UF60-3, UF60-4 and UF60-5 had MWCO values of 21, 19.5, 18.5, 17.5, and 13 kDa, respectively. From the result we can note that the higher rejection obtained for PEG. It is clear the MWCO was decreased when the CA content in casting solution increased. The rejection of PEG was related with the pore size of the membrane. The MWCO of the membranes between 13 and 21 kDa these data are in the range of ultrafiltration, hence the blended membrane is considered a UF membrane [38]. This result supports the high hydrophilicity and decrease of permeability of the polymer membranes.

3.5. Contact angle

The hydrophilicity of porous UF and surface properties was evaluated by water contact angle measurement. The results of contact angle were represented in Fig. 7. The contact angle are 65.5°, 59.15°, 57.66°, 35.43° and 31.12° for UF60-1, UF60-2, UF60-3, UF60-4 and UF60-5, respectively. The contact angle decreased with increased CA concentration from 15 to 21 (w/v) %. This result could be related to the hydrophilic nature of CA polymer [38]. The increase of membrane hydrophilicity enhanced the reactance fouling.

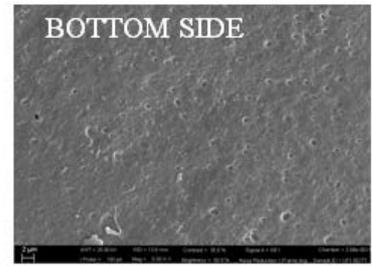
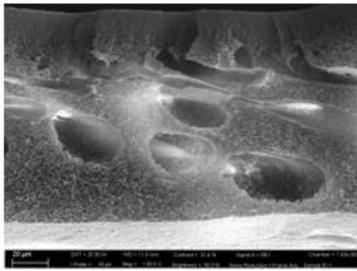
3.6. Water content

Water content is an important parameter for membrane characterization, the water content of the membrane is directly related to % porosity and water permeability of the membrane [35,39]. The measurements of water content of the synthesized membranes are reported in Table 2. We noted that the water content decreased from 76.1% to 61.32% when the concentration of CA polymer increased from 15 to 21 wt.%. This could be related to the fact that the membrane become more and more dense.

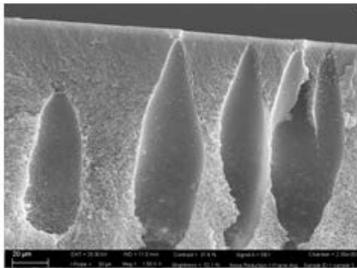
3.7. Pore size

The pore size of the synthesized membranes was measured in order to study how the polymer concentration affected the membrane properties. The results of this measure are summarized in Fig. 8. The pore sizes of the synthesized membrane were in the range 0.25–0.095 μm . The pore size was 0.25, 0.15 and 0.095 μm for UF-60-1, UF-60-3 and UF-60-5, respectively. The pore size depends on the polymer concentration; the increase of polymer concentration favors the reduction of the pore size. Hence, the decreasing of the pore size is explained the decreasing of permeability of the membranes.

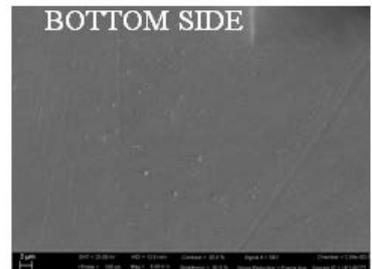
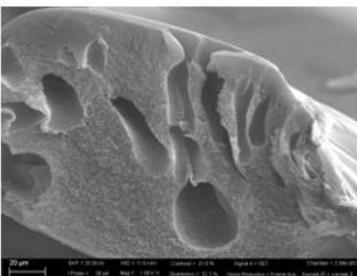
UF-60-1



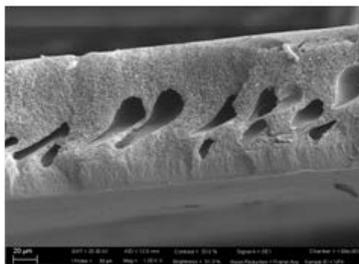
UF-60-2



UF-60-3



UF-60-4



UF-60-5

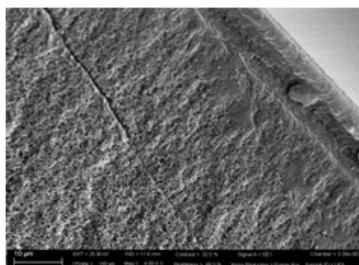


Fig. 4. SEM micrographs of CA membranes.

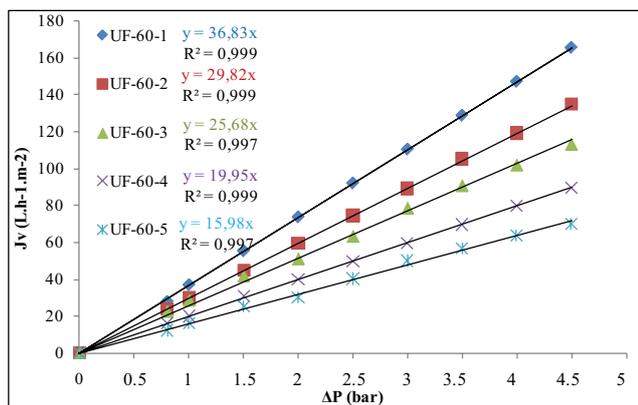


Fig. 5. Determination of the water permeability for the different CA membranes.

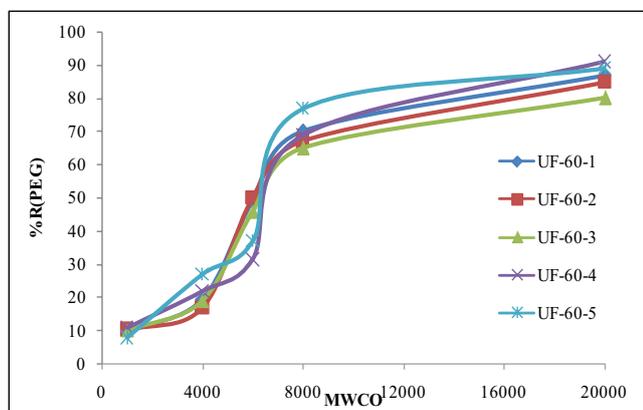


Fig. 6. Determination of MWCO for the different CA membranes.

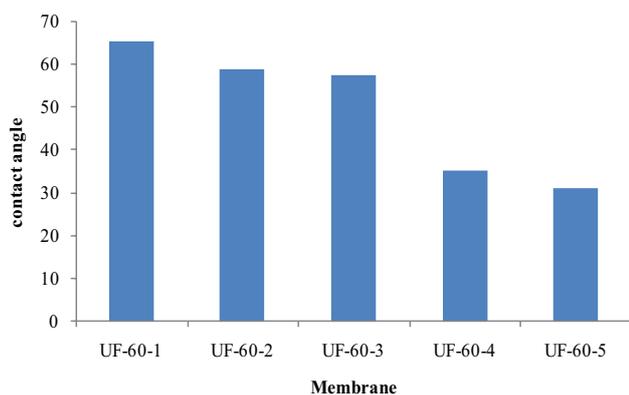


Fig. 7. Contact angle of the synthesized membranes.

3.8. Decontamination of water artificially contaminated by UPEC

Passage of bacterial cells through filter pores has been reported for a number of bacterial species. In this investigation, the effect on cell viability of pore size membrane is shown in Fig. 9. The results showed that the decrease of membrane pore size from 0.25 to 0.095 μm resulted in totally retention of UPEC. The results suggest that the bacteria size that is more than 0.45 μm ; but there are

Table 2

Determination of water content for the different CA membranes

Membrane	% Water content
UF-60-1	76.1
UF-60-2	73.15
UF-60-3	67.33
UF-60-4	65.47
UF-60-5	61.32

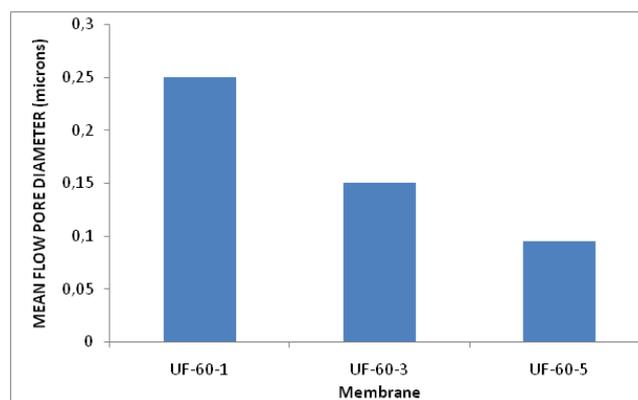


Fig. 8. Pore size for UF-60-1, UF-60-3, UF-60-5.

exceptions, perhaps stressed cells that can pass through 0.22 μm [40]. In addition, results indicated a continuous decrease of the bacterial load for the UF-60-1 and UF-60-2 membranes. However, no differences were observed between UF-60-3; UF-60-4 and UF-60-5; an absence of *E. coli* was detected after 5 min of retention time. The treatment by CA UF membranes typically results in the release of bacteria 4U-log to 8 U-log depending on retention time. The same disinfection performance obtained in this study had been previously reported by Ouyang et al. [41] via the use of combined chemical and physical filtration methods. Research by Saidi et al. [27] documented the production of virulent molecules from bacteria when irradiated by UV light. For this reason, treatment by membranes may be considered a safer alternative to UV treatment.

This research used these strains of UPEC commonly isolated in globally in countries such as Tunisia [18], Europe [42], India [43], USA [44] and Canada [45], Antibiotic-resistance epidemiological bacteria may be discharged from water or wastewater. The CA-UF synthesized membrane may limit the spread of these pathogenic bacteria.

4. Conclusions

In this study, ultrafiltration (UF) membranes were successfully synthesized using phase inversion method from casting solution consisting of CA used at increasing concentrations from 15 to 21 wt%. The performance of the membrane, for example, permeability, contact angle and water content was decreased with increasing of CA increased but the PEG rejection was increased at higher polymer in the membrane preparation. When applied to water

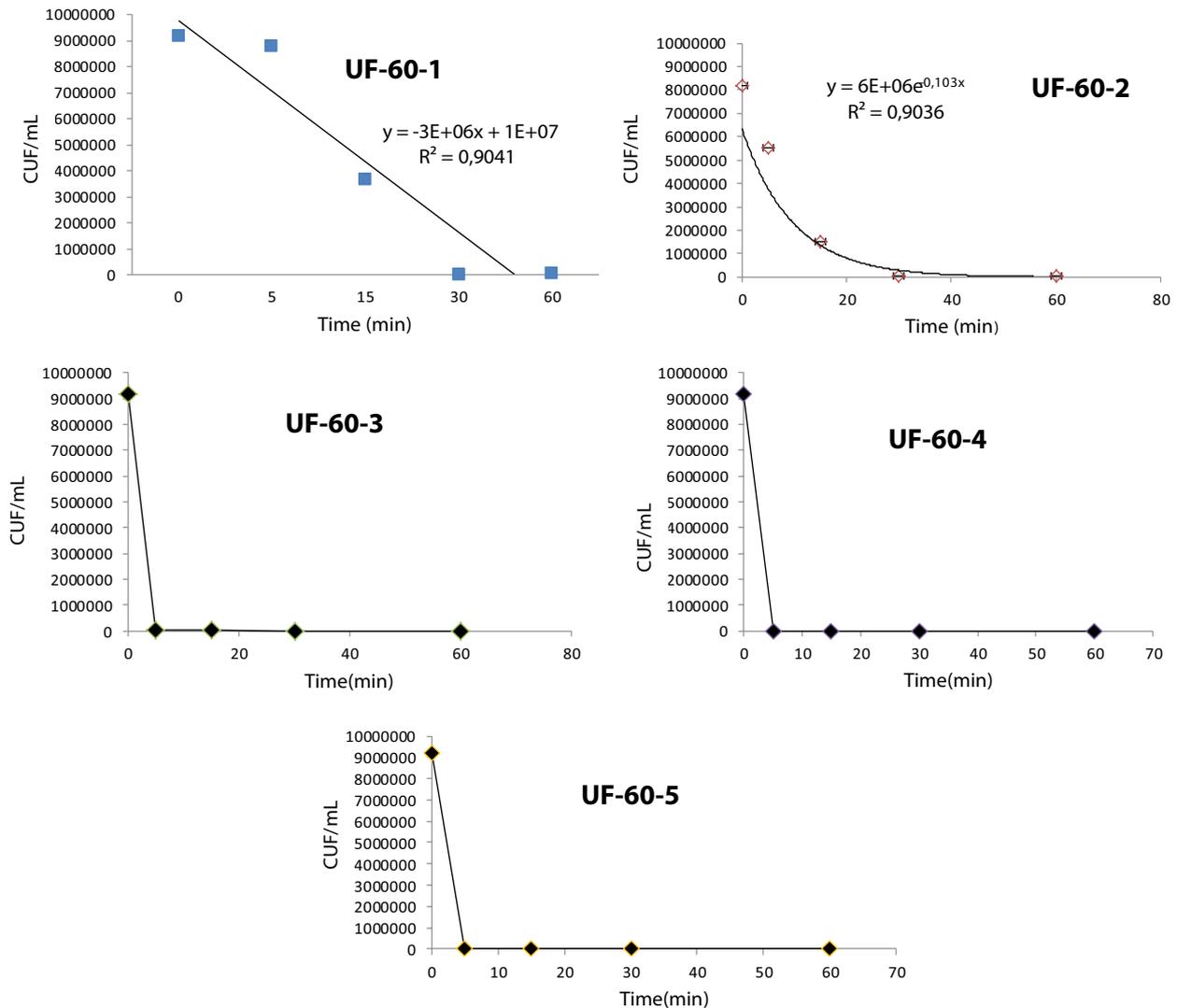


Fig. 9. Changes in the kinetics of disinfection of UPEC measured as a function of contact time (expressed in minutes) under the reactor. CFU: colony forming unit, Y axis: reduction N/N_0 , where N is the number of viable and cultivable cell after membrane treatment and N_0 is the number of viable and cultivable cell before membrane treatment. X axis present increasing time retention in the considered membrane reactor.

artificially contaminated by high load *E. coli* (10^8 bacteria/mL), the prepared membranes were an excellent tool for retaining *E. coli* and their performance can reach 8-U log. The retention and removal of epidemiological bacteria prior environment contamination can be a cost-effective public health strategy. Prepared membrane may be an interesting tool that may be applied in bio-medical or in pilot wastewater treatments to avoid the spread of pathogenic bacteria.

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Conflicts of interest

The authors declared no conflict of interest.

Symbols

- UF — Ultrafiltration
- CA — Cellulose acetate
- J — Flux, $L\ m^{-2}\ h^{-1}$
- P — Permeation ratio, %
- V — Volume of permeate pure water, L
- A — Effective area of the membrane, m^2
- Δt — Permeation time, h
- R — Rejection, %

C_p	—	Permeate concentration, g L ⁻¹
C_f	—	Feed concentration, g L ⁻¹
W_w	—	Weight of the wet membrane, g
W_d	—	Weight of the dry membrane, g

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