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Microbial Removal from Secondary Treated Wastewater Using a Hybrid System of Ultrafiltration and Reverse Osmosis

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Abstract: The efficiency of advanced membranes towards removal of general and specific microbes from wastewater was investigated. The treatment included a subsequent system of activated sludge, ultrafiltration (hollow fibre membranes with 100 kDa cut-off, and spiral wound membranes with 20 kDa cut-off), and RO (reverse osmosis). The removal evaluation of screened microbes present in treated wastewater showed that hollow fibre membrane rejected only 1 log (90% rejection) of the TPC (total microbial count), TC (total coliforms), and FC (faecal coliforms). A higher effectiveness was observed with spiral wound, removing 2-3 logs (99%-99.9%) of TPC and complete rejection of TC and FC. The RO system was successful in total rejection of all received bacteria. The removal evaluation of inoculated specific types of bacteria showed that the hollow membranes removed 2 logs (99%) of inoculated *E. coli* (10^7-10^8 cfu/mL inoculum), 2-3 logs (99%-99.9%) of *Enterococus* spp. (10^7-10^{10} cfu/mL inoculum), 1-2 logs (90%-99%) of *Salmonella* (10^8-10^{10} cfu/mL inoculum) and 1-2 logs (90%-99%) of *Shigella* (10^5-10^6 cfu/mL inoculum). The spiral wound was significantly efficient in rejecting further 3 logs of *E. coli*, 5 logs of *Enterococus* spp., 4 logs of *Salmonella*, and a complete rejection of all received bacteria was accomplished by RO membrane. The results indicate that Gram positive bacteria were removed much more efficiently compared to the Gram negative ones, the rationale behind such behaviour is based on cell walls elasticity.

Key words: Wastewater treatment, microbial load removal, ultrafiltration, reverse osmosis, filtration technology, microbial fouling.

1. Introduction

The Mediterranean Region is suffering from significant water shortage while the clean water claiming is everyday increasing. As a consequence, the search of new supplies along with protection of used sources becomes of major priority [1]. The need of water is growing rapidly in Palestine due to rapid population growth, urbanization and socioeconomic development. On the other hand, the increased production of wastewater creates a series of problems, including large-scale discharge of untreated wastewater, leaking of collected wastewater from sewer systems and cesspits, malfunction of wastewater treatment plants, and uncontrolled reuse of untreated wastewater by the irrigation sector [2]. The discharge of raw wastewater causes a major potential health hazard because part of it percolates into the ground affecting groundwater quality [3]. On the contrary, appropriate and sustainable sewage

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treatment systems can help to preserve biodiversity, maintain healthy ecosystems [4, 5], protect the public health [6-11], and provide water for unrestricted irrigation [12].

The choice of a wastewater treatment process for every particular application depends on the quality of the raw wastewater, the required quality of the treated water, and the economic resources available to sustain a treatment plant [13]. At the present time, most of wastewater treatment processes are undergoing continual and intensive development [12]. Generally, wastewater treatment is achieved by the removal of the suspended solids, dissolved chemicals, and pathogens. Therefore, wastewater treatment plants are built including a variety of physical, chemical and biological processes [14, 15]. The treatment processes of wastewater may comprise: (i) primary treatment, (ii) secondary treatment, and (iii) tertiary treatment. Advanced wastewater treatments consist of integrated series of steps. The primary treatment includes simple processes such as screening and grit removal to eliminate the gross solids, a sedimentation stage and a simple settling of solid materials [3]. In the secondary treatment sometimes the aeration and sedimentation are combined in a unique reactor [3] in which tricking filtration and activated sludge processes are carried out aiming at removal of biodegradable dissolved and dispersed organic matter by means of aerobic and anaerobic bio-reactions [16]. In the tertiary treatment specific pollutants, such as nitrogen and phosphorous or industrial pollutants, such as heavy metals, are removed.

The objective of tertiary treatment is to return wastewater to nearly its original quality [17], and it is used to lower as much as possible the concentration of BOD (biochemical oxygen demand), nitrogen, phosphorus, suspended and dissolved solids [15]. The tertiary treatment processes use chemical treatment, filtration, disinfection, membrane filtration, MBR (membrane bio-reactor), reverse osmosis and epuvalization [3, 18]. Chlorination is a crucial step in many treatment plants to kill microorganisms remaining after the biological treatment [3, 19]. Since many organic compounds can react with chlorine forming toxic compounds affecting the beneficial use of recycled water as in the case of trihalomethanes [3], the World Health Organization has established a guideline value of 5 mg/L for chlorine in drinking water [20].

Filtration by membrane technology is a relatively recent developing technology used to remove specific colloidal sized materials enabling the dissolved molecules pass through. There are different examples of membrane filtering systems such as MF (micro-filtration), UF (ultra-filtration) and RO (reverse osmosis) [15]. UF is a technique that has been used in various approaches since 1970s for the removal of microbes and viruses in water [21]. Currently, there are four common configurations of UF membranes: SW (spiral wound), HF (hollow fibres), tubular and plate and frame. Reverse osmosis membranes are capable for rejecting bacteria, salts, sugars, proteins and dyes. RO membranes like all other membranes are subjected to fouling by “cake layer” formation or scaling [22]. Integrating membrane systems (UF and RO membranes) with biological units such as activated sludge process may be a very efficient system in treating wastewater, rejecting microbes, and reducing membrane fouling without a need to chlorination.

Al-Quds University wastewater treatment plant collects a mixture of black, gray, and storm water, as well as wastewater (from certain laboratories). The treatment plant consists of a primary treatment (two stage primary settling basin), and secondary treatment (activated sludge with a hydraulic retention time of 16-20 h, followed by coagulation and chlorination). Then the secondary effluent is introduced to a sand filter before entering the ultrafiltration membrane, which consists of a UF HF (hollow fiber) with 100 kDa cut-off filters as pre polishing stage for the UF spiral wound with 20 kDa cut-off filters. The spiral

wound stage produces good water quality with less than 20 ppm BOD and less than 30 ppm TSS (total suspended solids) and free from fecal coliform bacteria, which makes the water suitable for non restricted irrigation. After ultrafiltration process, the effluent is filtered by activated carbon column followed by a reverse osmosis (advanced treatment). Then a blend of UF effluent and effluents of reverse osmosis with salt content similar to that of fresh water are used for irrigation. It is worth noting that before the installation of ultra filtration with hollow fiber membrane, the spiral wound membranes had suffered severely from fouling which rendered this process to be expensive and not feasible. This fouling was mostly eliminated and thus the operation of the system improved significantly with the introduction of the HF unit.

The objective of this study was to assess the effective removal of different types of bacteria using integrated wastewater treatment, including primary and activated sludge steps, and a subsequent hybrid system of ultrafiltration (hollow fibre and spiral wound UF membranes) and reverse osmosis.

2. Materials and Methods

2.1 Experimental Plant

The experiment for the evaluation of microbial removal from wastewater was conducted at Al-Quds University treatment plant. Wastewater to be treated in the Al-Quds plant is a mixture of black (from toilets), gray (from showers and sinks), and storm (rain) waters collected from the university campus, which hosts approximately 13,000 students and staff members in the day time. The plant includes a primary settling basin, followed by activated sludge unit, two ultrafiltration units and a reverse osmosis system.

The unit for the activated sludge process is able to treat up to 50 m^3/day of wastewater with 16-20 hours retention time. During aeration, the microorganisms metabolize the organic matter giving rise to a reduction of wastewater BOD. Wastewater is then treated with aluminium sulphate as coagulating agent to promote the removal of suspended solids. The secondary effluent is introduced into a sand filter before entering the first UF unit that houses HF membrane with 100 kDa cut-off. Then the permeate is introduced into the second UF unit that houses the SW membrane with 20 kDa cut-off. After the UF process, the effluent is subjected to an activated-carbon adsorbent followed by RO. The treated wastewater is collected for reuse in a special pond and the destruction of microbes is achieved by chlorination in the form of Trichlor discs. For our experimental purposes, a part of activated sludge treated wastewater was carried to the ultrafiltration system bypassing the chlorination step, which can damage the RO membranes. The ultrafiltration system used consists of two components: a hollow fibre UF unit having a capacity 36 m^3/day and a spiral wound UF unit with a capacity of 12 m^3/day according to the manufacturer's specifications. The hollow fibre unit is equipped with two pressure vessels that house the hollow fibre membranes having 100 kDa cut-off (AST technologies, Model No. 8000 WOUT_IN_8080, Israel). The spiral wound UF membranes consist of three layers: a polyester support web, a micro-porous polysulfone interlayer and ultra-thin barrier coating on the surface. The UF membrane type is NIROSOFT RM10-8, 8040 spiral wound. The MWCO (molecular weight cut-off) of the membrane is 20 kDa which is equivalent to 0.01 micron separation rate.

The UF compartment consists of a couple of 2 × 4 inch pressure vessels having a pressure resistance up to 150 psi. Each vessel holds two separate membranes. The RO membranes are manufactured from thin polyamide film having a working pH ranging 1-11 (model BW30-4040 by DOW Filmtec, USA). The RO compartment consists of 1 × 4 inch pressure vessel of composite material with a pressure resistance up to 400 psi. The vessel holds two 4 inches RO membranes. An anti-scaling commercial product (NCS-106-FG, mainly containing phosphonic acid disodium salt) is

continuously dosed to the RO feed at a concentration of 4 ppm in order to prevent the deposition of divalent ions. The RO system is designed to remove major ions and heavy metals with a permeate capacity of 0.5 m^3/h (12 m^3/day).

During the experiment, the secondary treated effluent was pumped to the hollow fibre ultra-filtration unit, then to the spiral wound ultra-filtration unit. The UF permeate was collected in a special tank and used to feed the RO unit.

2.2 Screening of Microbial Parameters

For screening purposes, five samples were monthly collected. Each sampling included raw wastewater, waters after activated sludge process, after hollow fibre UF, after spiral wound UF, and finally after reverse osmosis. The microbial parameters screened were: total microbial count, total coliform and faecal coliform contents, and finally *Escherichia coli* and *Enterococus* spp. as specific bacterial charge.

Wastewater carried from the Al-Quds plant was free from *Shigella* and *Salmonella*. This indicates that the university community was free from diseases caused by these bacteria. However, these types of bacteria may be found in the wastewater from hospitals and health centres or domestic wastewater; for this reason, *Shigella* spp. and *Salmonella* spp. were considered in modelling experiments as depicted below.

2.3 Modelling Experiment

In order to assess the efficiency of the hybrid system of ultrafiltration and reverse osmosis, four model bacteria were used in the modelling experiment: three Gram negative (*E coli*, *Salmonella* spp., and *Shigella* spp.) and a Gram positive (*Enterococus* spp.). Microbes selected for this investigation were grown in the laboratory under complete sterile technique, and separately inoculated using specific counts in 500-L fresh water contained in 1,500-L plastic tanks. The inoculated water was passed through the hollow fibre UF unit followed by spiral wound UF unit, and finally through the RO system.

Microbial analyses were performed on samples collected from the inoculation containers, after the filtration with the hollow fibre membranes, from the retentate of hollow fibre UF, after the filtration with the spiral wound membranes, from the retentate of spiral wound UF, after reverse osmosis, and from the retentate of reverse osmosis.

2.4 Isolation, Identification and Counting of Bacteria

Counting and identification of all types of screened and model microbes were done according to standard methods for examination of water and wastewater [23]. Bacteria in the samples were counted directly after sampling under complete sterile technique. Serial dilutions with sterile peptone water were done whenever it was required. The growth media, growing conditions and colonies characteristics of tested microbes are listed in Table 1.

A no-treated wastewater sample was filtered through a 0.45 μm filter under complete sterile conditions. The filter was then placed on sterile M-FC

Table 1 Growth media, suitable conditions for bacterial tests, and colony characteristic colour of tested bacteria.

Bacterial type	Medium	Incubation temperature (°C)	Incubation time (h)	Colony colour
Total coliform	M-Endo agar	37	24	Green metallic sheen
Faecal coliform	M-FC agar base	44.5	24	Dark blue
E. coli	m-TEC agar	35.5 (0-2 h) 44.5 (2-22 h)	22	Yellow to brown
E. coli	MacConkey agar	37	24	Pink
TPC (total microbial count)	Plate count agar	37	48	Colourless
Faecal enterococci	M-enterococcus agar	37	48	Pink to red
Salmonella spp.	BG and XLD agar	37	24	Pink on BG, black on XLD
Shigella spp.	Hektoen and XLD agar	37	24	Green on Hektoen, pink-red on XLD

agar (267720, Difco) plates and then was incubated for 24 h at 44.5 °C; typical dark blue colonies were picked as faecal coliform bacteria. From colonies of faecal coliforms *E. coli* bacteria were isolated as yellow to brown colonies when grown on m-TEC agar (M1391, Himedia, India) and as pink colonies when cultured on MacConkey agar (M008S, Himedia, India). *Enterococus* spp. bacteria were isolated from fresh raw wastewater cultured on m-Enterococus agar (274620, Difco) as selective media. Streaked plates were incubated at 37 ºC for 48 h, then catalase and oxidase tests were done for all observed pink to red colonies as a confirmative test. Catalase and oxidase negative tests confirmed the presence of *Enterococcus* spp. bacteria, which were recognized as pink colonies with dark pink point at the centre. *Salmonella* and *Shigella* bacterial cells were obtained as pure cultures from stored bacterial stocks of Al-Quds University medical laboratories because they were absent in the wastewater produced in the period of modelling experiments. *Salmonella* was confirmed as black colonies when grown on XLD agar (M031, Himedia, India) and as pink colonies when grown on BG agar. *Shigella* was confirmed as pink to red colonies when grown on XLD agar and as green colonies on Hektoen agar (M467, Himedia, India).

2.5 Determination of the Bacterial Growth Curves

In order to use fresh bacterial suspensions at the log phase of their growth curves, a bacterial growth curve was performed for each type of bacterium under investigation. Furthermore, in order to predict the concentration of any bacterial suspension, plots of bacterial count vs. optical density were drawn (Fig. 1).

In 250 mL modified Erlenmeyer flasks (supplied with a test tube to measure the optical density without opening the flask) 150 mL sterile nutrient broth were prepared. For each bacterial growth curve, a typical colony of one model bacteria from a fresh pure culture (Table 1) was transferred into the flask under a complete sterile technique. All the growth flasks were capped with sterile cotton, and then incubated in a shaking incubator under specific growth temperature depending on the studied bacterium (Table 1). The absorbances of the bacterial suspensions in the growth flasks were measured at wavelength 545 nm before incubation to set the zero starting value. The optical density of the bacterial suspension was measured after different times of incubation depending on each type of bacterium. Up to 10 serial dilutions were settled on according to the value of the optical density of the bacterial suspension. For each dilution, 1 mL was transferred in duplicate to a 90 mm Petri plates then 15 mL of sterile nutrients' milted agar were poured into the plate containing the sample (pour plate method). After the plates solidified, they were incubated using temperatures and times as referred in Table 1. Bacterial counts were recorded and linear relationships between the logarithmic values of the bacterial counts and the measured optical densities at each sampling time were drawn as shown in Fig. 1. For mathematical reasons concerning the Log-scale zero bacterial counts were replaced by 1.

2.6 Preparation of Inoculants

For each type of bacterial model, 20 nutrient agar plates (90 mm diameter) were streaked with the pure culture of bacterial colonies to obtain a confluent growth of each type of bacterium. Streaked plates were incubated 24 h at a temperature (Table 1) specific for each type of bacterium. To harvest bacteria cells, 5 mL of sterile nutrient broth was poured on each plate having a significant bacterial growth, and then the bacterial colonies were collected using a L-shaped sterile glass rod by moving it gently in a round movement to gather a thick bacterial suspension in a 3-L sterile bottle. Using the growth curve obtained for each type of bacterial model (Fig. 1), the bacterial suspension was adjusted to the optimal optical density, which corresponds to the required bacterial count (Table 2). Three litres of bacterial suspension at the required optical density

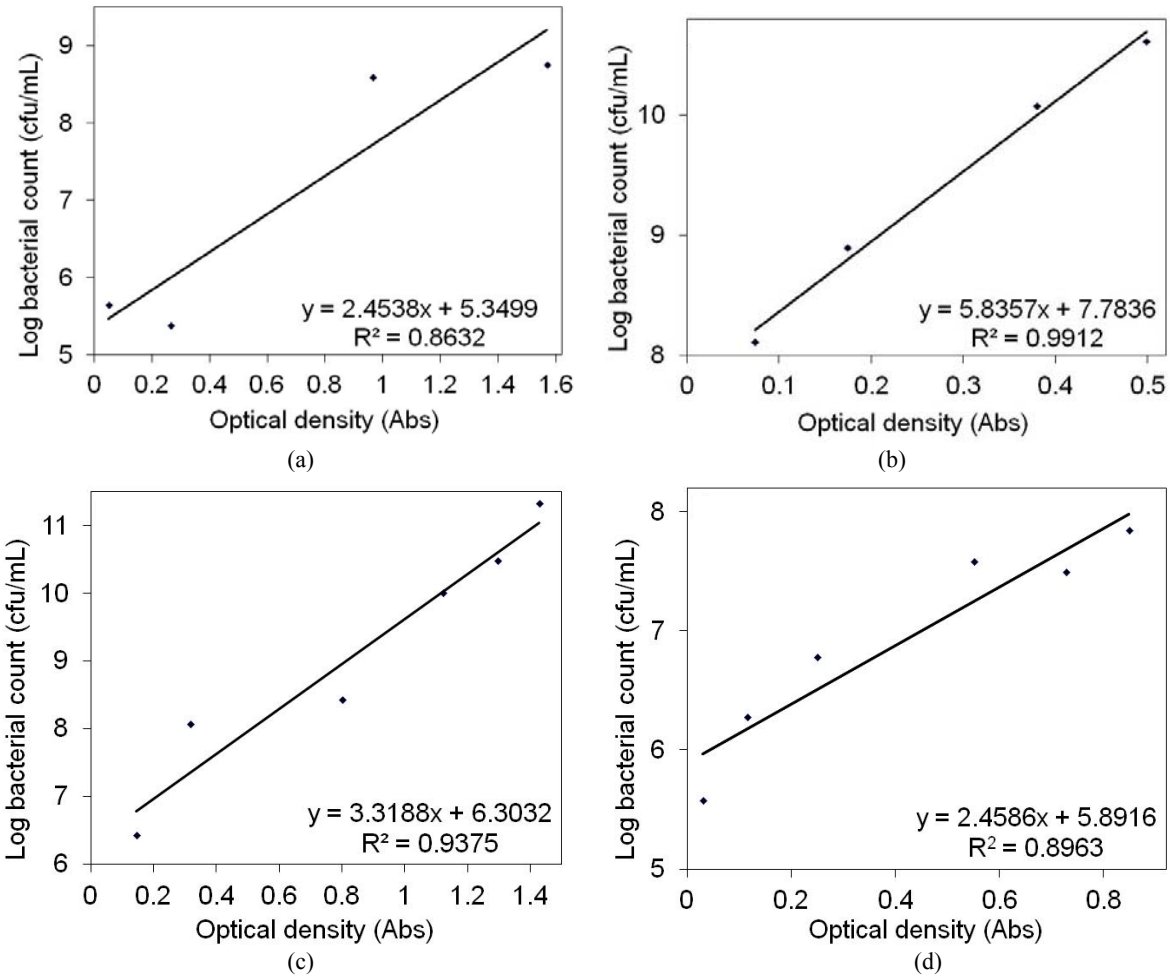

Fig. 1 Linear relationships between logarithmic bacterial count and optical density (545 nm) of (a) *E. coli*; (b) *Enterococcus* spp.; (c) *Salmonella*; and (d) *Shigella* cultures at the log phase of their growth curves (8-10 hours of incubation).

Table 2 Bacterial counts settled by the corresponding optical density (Abs.) for each type of bacterial model.

Type of bacteria	Bacterial count (cfu/mL)	Optical density (absorbance at 545 nm)
E. coli	10^7	0.75
Salmonella spp.	10^7	0.75
Shigella spp.	10^8	0.55
Enterococcus spp.	10^7	0.35

were prepared and inoculated in a tank filled with 500 L fresh water, stirred with a clean wooden stick to have a homogenous bacterial-water liquid to feed the hybrid system of ultrafiltration and reverse osmosis. Bacterial counts in the 3-L bacterial suspension and in the inoculated 500 L fresh water in the plastic tank were determined using pour plate method. Samples were taken from the mentioned sampling sites after 10 minutes of operation, collected in sterile bottles, and sent directly to the laboratory for bacterial count. The inoculation experiment was done three times in different days with different inoculum counts for each bacterial model separately.

3. Results

3.1 Screening of Microbial Parameters

3.1.1 Activated Sludge Process

Activated sludge process (preliminary to the UF and RO filters) without chlorination reduced the total

microbial count 1 log (90% removal) in most of the samples, and 2 logs (99% removal) in some of them with respect to the initial microbial population counted in the row wastewater (Table 3).

In particular, total coliform charge (Table 4) was reduced after passing the activated sludge process at least 1 log (90% removal), and sometime 2-3 logs (99%-99.9% removal) without chlorination. Faecal coliform load (Table 5) was reduced 1-2 logs.

3.1.2 Hollow Fibre Ultrafiltration

Our results (Table 3) show that the hollow fibre UF was not sufficiently efficient in the reduction of total microbial count of the unchlorinated effluent carried from the activated sludge biological treatment. Only some samples showed 1-2 logs reduction (90%-99%) of the total microbial count. In terms of total coliform bacteria, the reduction was 1-2 logs and reached to 4 logs in some cases (99.99%). But in some sampling dates, there was no significant reduction (Table 4). Although hollow fibre UF membrane completely eliminated FC bacteria in some samples (10^3-10^5 cfu/mL), it rejected only 90% of the faecal coliforms in some other samples (Table 5). Therefore, the hollow fibre ultrafiltration membrane was not satisfactorily efficient in the reduction of the microbial indicators under investigation (TPC, TC and FC). This means that the use of hollow fibre as a sole UF membrane is not acceptable to eliminate the microbial health hazards, and must be coupled with further treatment processes.

Table 3 Performance of the activated sludge process (the effluent is not chlorinated), HF-UF (hollow fibre ultrafiltration), SW-UF (spiral wound ultrafiltration) and RO for the reduction of TPC, all data are Log transformed. The effluent of one compartment is the influent of the subsequent one.

Date	Days	Activated sludge		HF-UF permeate	SW-UF permeate	RO permeate
		Influent	Effluent			
July 15, 2008	0	5.6	4.7	4.0	3.5	0.2
August 05, 2008	35	5.4	4.9	4.1	2.3	0.0
September 02, 2008	67	5.2	4.8	4.2	2.1	0.0
October 07, 2008	102	5.5	4.7	4.4	2.4	0.0
November 10, 2008	135	5.6	4.8	4.3	2.3	0.0
November 24, 2008	149	5.2	3.7	1.0	0.0	0.0
January 20, 2009	205	5.0	3.6	2.8	2.0	0.0
February 09, 2009	224	4.3	3.7	2.7	1.7	0.0
March 17, 2009	261	4.7	4.3	2.5	1.3	0.0
April 14, 2009	288	7.3	7.0	6.3	5.6	0.0
May 19, 2009	323	7.0	6.6	4.6	3.3	0.0
June 09, 2009	343	6.8	6.2	4.6	4.0	0.3
July 14, 2009	378	7.6	6.3	4.0	2.8	0.2
July 28, 2009	392	6.0	5.3	3.8	3.0	0.0
August 30, 2009	432	7.4	5.8	4.6	3.3	0.0
October 07, 2009	468	7.9	5.3	4.5	2.5	0.0
November 04, 2009	495	6.6	5.1	4.8	2.0	0.2
December 07, 2009	528	6.0	4.7	4.3	2.2	0.0
January 31, 2010	582	6.0	5.2	4.5	2.7	0.1
March 30, 2010	640	8.0	6.0	4.3	1.3	0.0
April 27, 2010	667	7.6	6.6	3.0	1.4	0.0
June 29, 2010	729	7.2	6.3	4.8	2.7	0.0
July 28, 2010	758	6.5	5.8	5.0	2.3	0.2
August 25, 2010	785	7.3	5.3	5.3	3.3	0.0

Table 4 Performance of the activated sludge process (the effluent is not chlorinated), HF-UF, SW-UF and RO for the reduction of TC (total coliform) bacteria, all data are Log transformed. The effluent of one compartment is the influent of the subsequent one.

Date	Days	Activated sludge		HF-UF	SW-UF	RO
		Influent	Effluent	permeate	permeate	permeate
July 15, 2008	0	4.8	3.5	3.1	2.5	0.9
August 05, 2008	35	5.0	3.7	3.3	1.7	0.0
September 02, 2008	67	4.8	3.6	3.2	1.5	0.0
October 07, 2008	102	4.8	3.8	3.5	0.0	0.0
November 10, 2008	135	4.5	3.7	1.3	0.0	0.0
November 24, 2008	149	4.3	4.0	2.5	0.0	0.0
January 20, 2009	205	4.4	4.9	2.7	0.0	0.0
February 09, 2009	224	3.9	3.3	2.3	0.0	0.0
March 17, 2009	261	4.6	4.5	2.7	0.3	0.0
April 14, 2009	288	6.1	5.8	4.3	2.5	0.0
May 19, 2009	323	5.5	4.8	3.6	0.3	0.0
Jun. 09, 2009	343	5.8	5.3	3.2	0.0	0.0
July 14, 2009	378	6.5	5.5	1.0	0.0	0.0
July 28, 2009	392	5.1	4.3	1.7	0.0	0.0
August 30, 2009	432	7.2	4.7	2.7	0.0	0.0
October 07, 2009	468	6.8	4.1	1.4	0.0	0.0
November 04, 2009	495	5.7	4.9	4.3	0.5	0.0
December 07, 2009	528	4.0	3.5	2.8	0.0	0.0
January 31, 2010	582	5.0	3.5	3.1	0.5	0.0
March 30, 2010	640	5.7	4.1	4.0	1.7	0.0
April 27, 2010	667	5.8	5.5	0.0	0.0	0.0
June 29, 2010	729	6.5	5.4	0.0	0.0	0.0
July 28, 2010	758	6.1	5.7	3.3	0.0	0.0
August 25, 2010	785	6.9	4.3	2.1	0.7	0.0

Table 5 Performance of the activated sludge process (the effluent is not chlorinated), HF-UF, SW-UF and RO for the reduction of FC (faecal coliform) bacteria, all data are Log transformed. The effluent of one compartment is the influent of the subsequent one.

Date	Days	Activated sludge		HF-UF	SW-UF	RO
		Influent	Effluent	permeate	permeate	permeate
July 15, 2008	0	4.6	2.6	1.4	0.0	0.0
August 05, 2008	35	4.7	2.8	1.9	0.0	0.0
September 02, 2008	67	4.6	2.9	2.0	0.0	0.0
October 07, 2008	102	4.6	2.9	2.6	0.0	0.0
November 10, 2008	135	3.8	2.3	1.8	0.0	0.0
November 24, 2008	149	4.0	3.6	1.0	0.0	0.0
January 20, 2009	205	3.9	2.7	2.3	0.0	0.0
February 09, 2009	224	3.3	3.0	2.0	0.0	0.0
March 17, 2009	261	4.6	4.3	1.7	0.0	0.0
April 14, 2009	288	5.3	4.6	4.0	2.0	0.0
May 19, 2009	323	5.3	4.6	3.2	0.0	0.0
June 09, 2009	343	5.2	4.7	2.8	0.0	0.0
July 14, 2009	378	6.3	5.1	1.0	0.5	0.0
July 28, 2009	392	5.0	4.1	1.0	0.0	0.0
August 30, 2009	432	6.1	4.5	2.5	0.0	0.0
October 07, 2009	468	4.8	2.8	0.0	0.0	0.0
November 04, 2009	495	5.3	4.8	3.5	0.0	0.0
December 07, 2009	528	3.7	3.1	2.7	0.0	0.0
January 31, 2010	582	4.9	2.8	0.0	0.0	0.0
March 30, 2010	640	5.5	4.3	0.0	0.0	0.0
April 27, 2010	667	5.5	5.0	0.0	0.0	0.0
June 29, 2010	729	5.8	5.3	0.0	0.0	0.0
July 28, 2010	758	6.0	4.1	3.2	0.0	0.0
August 25, 2010	785	6.8	4.1	0.0	0.0	0.0

In addition, the rejection extent of *E. coli* by hollow fibre UF system was low (from 600 cfu/mL to 300 cfu/mL), the retentate of the hollow fibre UF contained more *E. coli* than the amount rejected by the filter, because this liquid is easily subjected to contamination due to the accumulation of all rejected particles and microbes (Table 6) in the hosting container. Although the wastewater under investigation contained relatively low number of *Enterococus* spp., the reduction of this bacterial species fluctuated from no reduction, half reduction, and two third reduction.

3.1.3 Spiral Wound Ultra-Filtration

After two pre-treatment processes of the row wastewater (activated sludge followed by hollow fibre ultrafiltration), spiral wound ultrafiltration membrane was highly efficient in rejecting most of the total microbial count, total coliform bacteria, and faecal coliform bacteria (Tables 3-5). It reduced the total microbial count by 2-3 logs (99%-99.9% rejection), total coliform bacteria by at least 1-2 logs in some samples, and total bacterial elimination was achieved in other samples. In addition, complete elimination of all faecal coliform bacteria in most samples was attained.

Spiral wound UF was very efficient in reducing the number of *E. coli*, which was passed to undetectable count (nearly to zero). The rejected bacteria from the spiral wound UF system was found in its retentate. The presence of *E. coli* in the retentate, which showed a total electric conductivity of 5 mS/cm, indicates that its salinity is not sufficiently enough to provide instant death of *E. coli*, and this retentate must be treated before its use in irrigation or dumping. The spiral wound decreased the *Enterococus* spp. content as well as observed for the hollow fibre membrane.

3.1.4 Reverse Osmosis

In the majority of the samples collected during the experimental period, reverse osmosis rejected all the total microbial counts remained after two ultrafiltration treatments (Table 3), although the total microbial load ranged 2-3 logs values in most of the entered samples and in some samples was 4 and 5 logs values. In very few samples, some microbial cells were detected (2-3 cfu/mL) after RO process. In terms of TC and FC bacteria, the reverse osmosis system rejected the few bacteria passed through the spiral wound UF membrane.

Since the spiral wound UF reduced the number of *E. coli* to undetectable counts, the influent to the RO system was approximately free of *E. coli*, and consequently the RO permeate did not contain this type of bacteria. Removal of *Enterococus* spp. by the RO membrane was complete (Table 7). This type of bacteria was not found in any sample of the retentate of the spiral wound and RO filters.

Table 6 Performance of the hollow fibre ultrafiltration, spiral wound ultrafiltration, and reverse osmosis for the reduction of *E. coli* (cfu/mL).

Date	Hollow fibre			Spiral wound			Reverse osmosis		
	Influent	Retentate	Permeate	Influent	Retentate	Permeate	Influent	Retentate	Permeate
January 06, 2010	600	500	600	600	1,300	2	2	0	0
January11, 2010	1,000	1,000	0	0	0	0	0	0	0
February 21, 2010	600	410	190	190	0	0	0	0	0

Table 7 Performance of the hollow fibre ultrafiltration, spiral wound ultrafiltration and reverse osmosis for the reduction of *Enterococus* spp. (cfu/mL).

Date	Hollow fibre			Spiral wound			Reverse osmosis		
	Influent	Retentate	Permeate	Influent	Retentate	Permeate	Influent	Retentate	Permeate
January 06, 2010	200	100	90	90	0	1	1	0	0
January 11, 2010	70	80	60	60	0	50	50	0	0
February 21, 2010	135	90	70	70	0	30	30	0	0

3.2 Modelling Experiments

The efficiency of the membranes (hollow fibre UF, spiral wound UF, and reverse osmosis) was evaluated in terms of rejecting different bacteria used as models and inoculated following a designed count. Two types of bacteria in terms of their Gram stain reaction were used: Gram negative (*Eschirechia coli*, *Salmonella*, and *Shigella*) and Gram positive bacteria (*Enterococus* spp.). Each type of bacteria was passed separately and sequentially through the system of the two ultra-filtration membranes and RO filter.

3.2.1 Rejection of Inoculated *E. coli* (Gram Negative Bacteria)

Fig. 2a represents the bacterial counts influent and permeate of the hollow fibre UF membrane for three independent trials in which *E. coli* was inoculated at 10^6, 10^7 and 10^8 cfu/mL, respectively. This count was reduced of only one to two log values (90%-99% rejection) in the effluent of this membrane, and all rejected bacteria were detected in the retentate produced by the filtering system.

The permeate of the hollow fibre, whose *E. coli* charge was ranging about 10^5 cfu/mL for all the three trials experimented, was carried to the spiral wound unit, which produced a filtered water having a bacterial charge reduced to about 10^2 cfu/mL (Fig. 2b). The *E. coli* was diminished of 3 logs values showing a high efficiency of the spiral wound in cutting down the elevated concentration of the *E. coli* inoculum (99.9% reduction). This membrane was found to be much more efficient than the hollow fibre membrane. Also in this case the rejected *E. coli* was detected in the retentate of spiral wound unit.

Fig. 2c illustrates the efficiency of RO system in terms of rejecting *E. coli* after its loading with the effluent of the spiral wound unit having a bacterial charge of about 10^2 cfu/mL. This stage was highly efficient producing an effluent practically free of such bacteria. In the first and third trials of this experiment, the effluent of the RO system showed a few count of *E.*

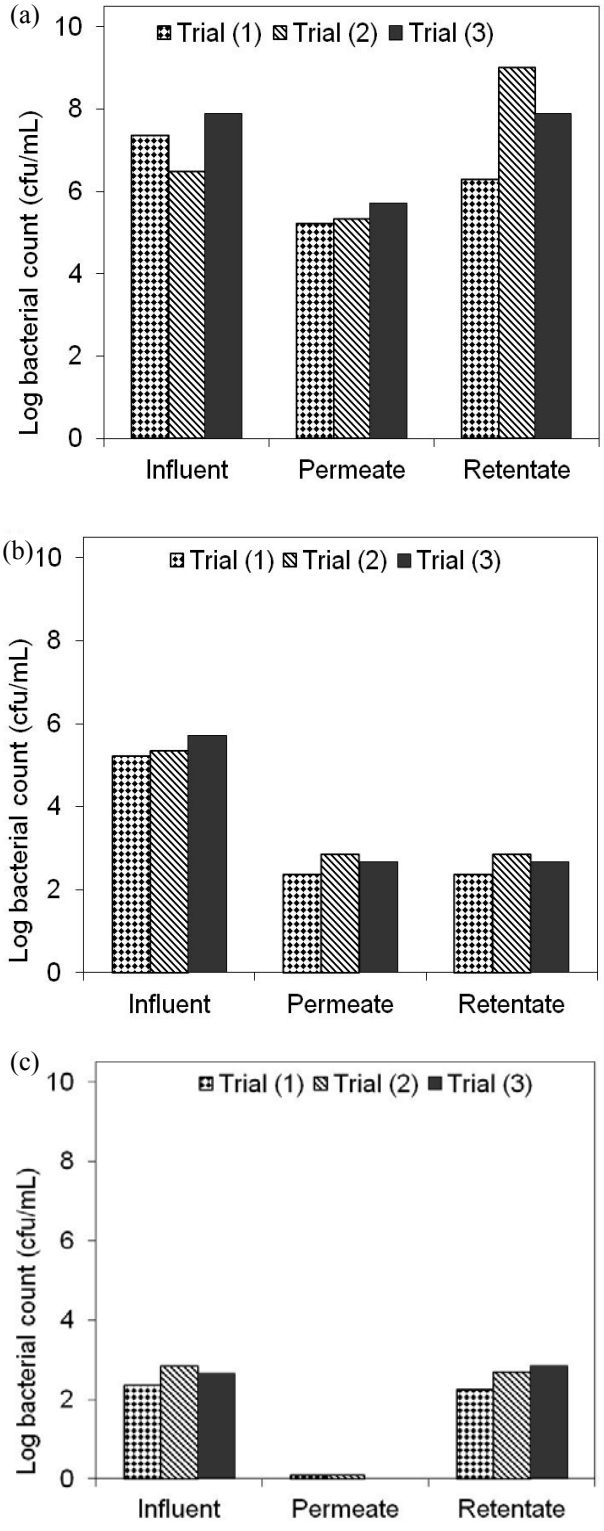

Fig. 2 Difference in counts of inoculated *E. coli* between influent and permeate inoculum passed through the (a) hollow fibre ultrafiltration, (b) spiral wound and (c) RO units.

coli (1-3 cfu/mL) taking in consideration that the treatment system is not working under sterile conditions and could be subjected to contamination.

3.2.2 Rejection of Inoculated *Enterococcus* spp. (Gram Positive Bacteria)

Results obtained from the three observations of the model experiment concerning *Enterococu* spp. (Fig. 3a) demonstrated that the inoculated bacterial counts (10^7, 10^9 and 10^{11} cfu/mL in the respective trials) were reduced after passing through the hollow fibre UF membrane to 10^4, 10^4 and 10^8 cfu/mL respectively. The data revealed that the hollow fibre reduced the inoculated bacterial count of about 3-5 logs values (about 99.9%-99.999% rejection). Hollow fibre was more efficient in rejecting *Enterococus* spp. than *E. coli*, taking into account that the former is a Gram positive bacterium with tough cell wall compared to the latter, which is Gram negative having elastic cell wall.

The count of *Enterococus* spp. that was carried from the hollow fibre to the spiral wound membrane was about 10^4 cfu/mL in both the first and second trials and 10^8 cfu/mL in the third inoculation trial (Fig. 3b). The spiral wound membrane was further efficient reducing of 3 logs values the concentration of the microbe under investigation in the first trial, 2 logs values in the second trial and 5 logs values in the last trial (99.9%-99.999% reduction). The *Enterococus* spp. bacterial cell is Gram positive with a hard cell wall, for this reason, the spiral wound membrane was able to reject these bacteria much more efficiently than *E. coli*, which is Gram negative having soft cell wall. The effluent generated after spiral wound, which was used as the RO membrane influent, was loaded by about 200 cfu/mL *Enterococus* spp. in both the second and third trials, besides the bacterial charge was practically zero in the first trial (Fig. 3c). Bacterial cells were almost completely rejected after RO filtration showing that the use of a subsequent filtration system in which the two types of UF membranes are followed by a RO membrane can be highly recommended when it is necessary to eliminate a significant load of *Enterococus* spp..

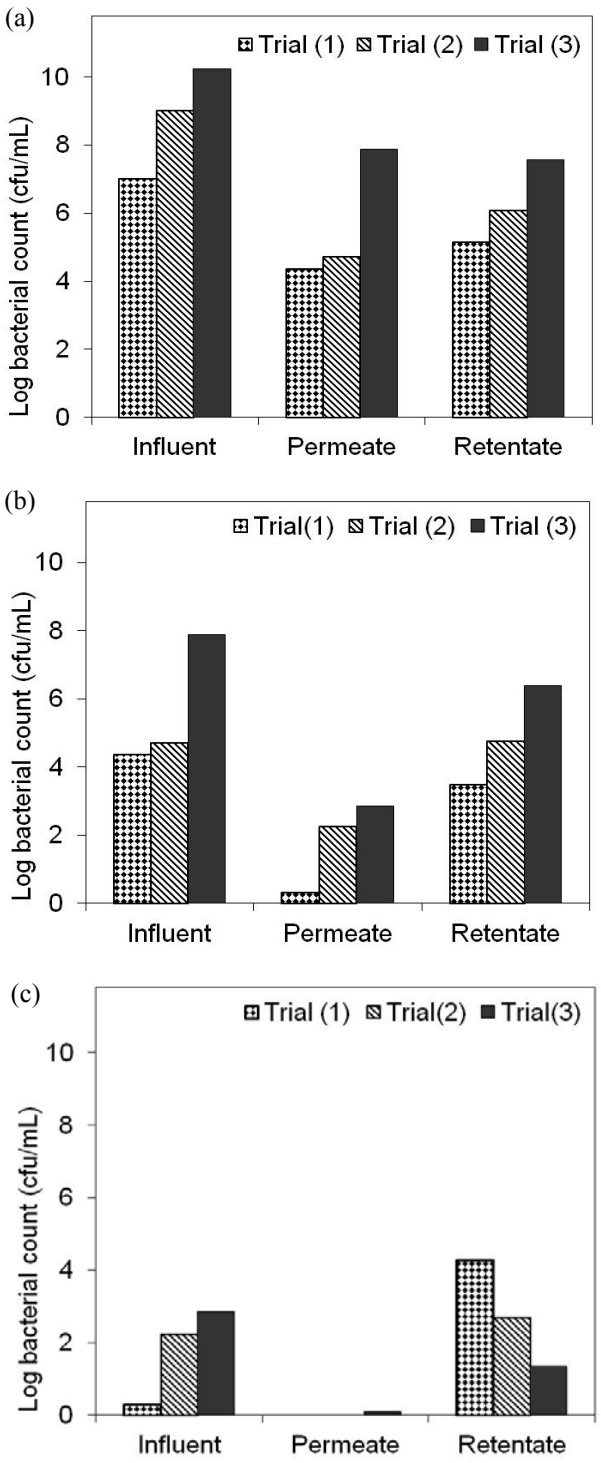

Fig. 3 Difference in count of inoculated *Enterococus* spp. between influent and permeate inoculum passed through the (a) hollow fibre ultrafiltration, (b) spiral wound and (c) RO units.

3.2.3 Rejection of Inoculated *Salmonella* spp. (Gram Negative Bacteria)

The hollow fibre unit was inefficient in rejecting inoculated *Salmonella* spp. (Fig. 4a). The inoculated bacterial concentrations that enter the hollow fibre membrane in the first and second trials were 10^8 cfu/mL and were reduced to 10^6 and 10^7 cfu/mL, respectively, whereas the bacterial count was reduced from 10^{10} to 10^8 cfu/mL in the third trial. Results indicated that the reduction of *Salmonella* was about 1 to 2 logs values (90%-99% rejection), which were close to the outcome obtained for *E. coli* (Fig. 2a) as both types of bacteria are Gram negative.

In the first trial, the spiral wound membrane was able to reduce the number of *Salmonella* spp. from 10^7 to 10^2 cfu/mL (99.99% rejection), while the inoculated count in the second trial was 10^6 cfu/mL and was reduced to 10^3 cfu/mL (99.9% rejection). The third trial showed that spiral wound membrane was capable to reduce the inoculated *Salmonella* of 5 logs values when the bacterial inoculum was more concentrated (from 10^8 to 10^3, about 99.999% rejection) as depicted in Fig. 4b. Altogether, the spiral wound membrane showed a potential of bacterial reduction ranging from 99.99% to 99.999% of the bacterial load investigated.

Finally, the RO was the most efficient system for the reduction of the inoculated *Salmonella* spp. (Fig. 4c). The spiral wound effluent had a bacterial count of 200-700 cfu/mL, which passing through the RO membrane was reduced in the all trials to undetectable count.

3.2.4 Rejection of Inoculated *Shigella* spp. (Gram Negative Bacteria)

The examination of removal effectiveness of inoculated *Shigella* by the hollow fibre membrane (Fig. 5a) verified that this membrane is inefficient in the rejection of this pathogen. The inoculated bacteria in the first trial were 5×10^5 cfu/mL and were reduced to 2×10^3 cfu/mL after passing through this membrane. Inoculated and released bacterial concentrations were 5×10^4 cfu/mL influent to 5×10^3 cfu/mL permeate and

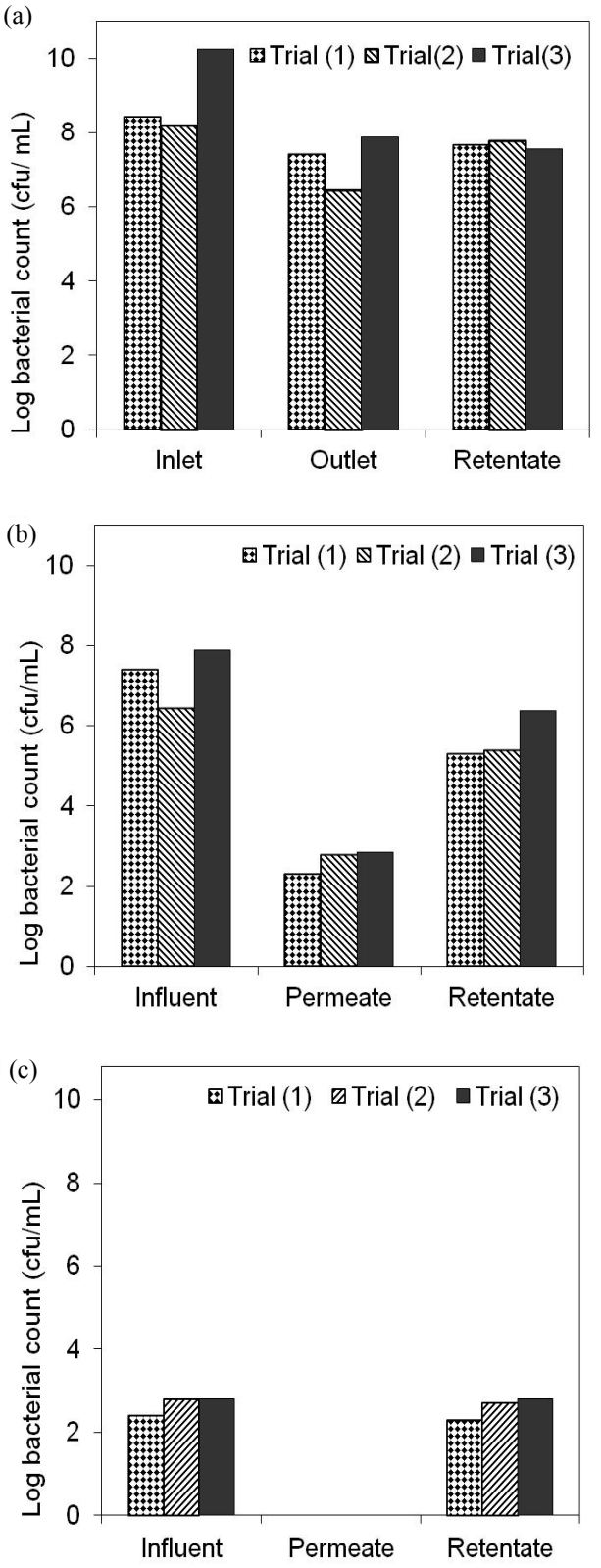

Fig. 4 Difference in count of inoculated *Salmonella* spp. between influent and permeate inoculum passed through the (a) hollow fibre ultrafiltration, (b) spiral wound and (c) RO units.

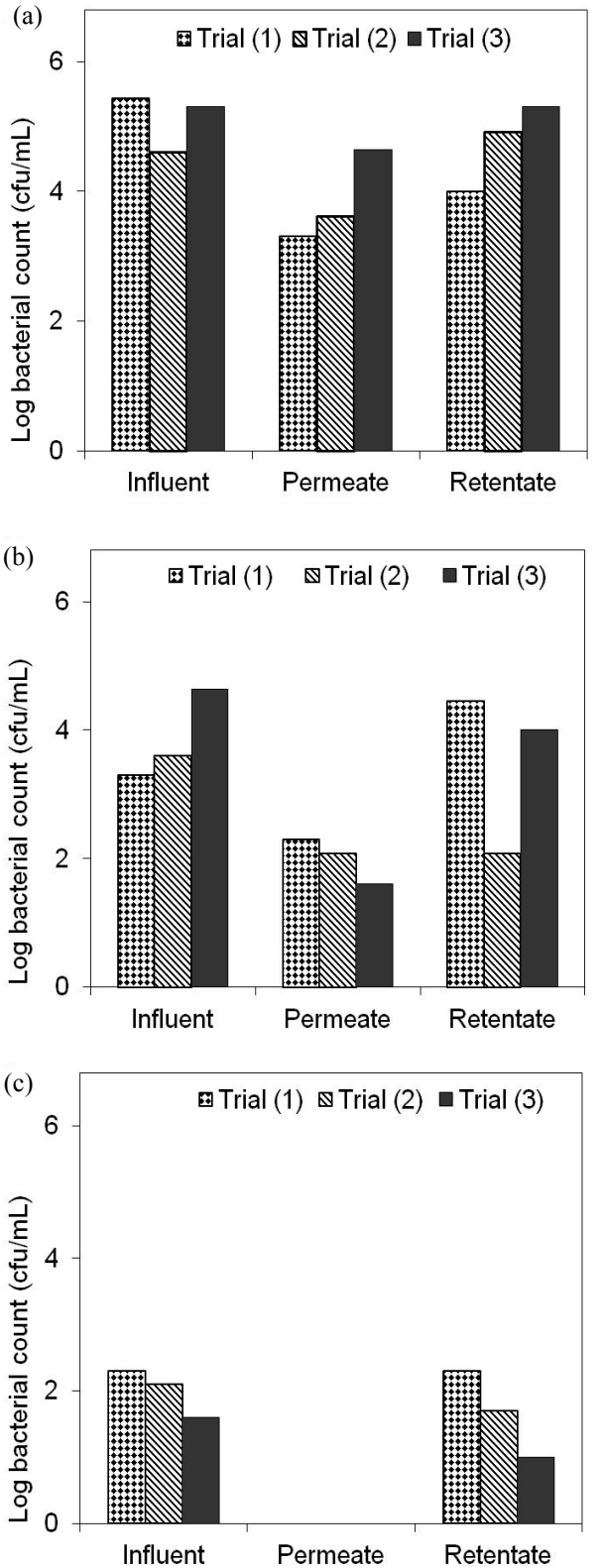

Fig. 5 Difference in count of inoculated *Shigella* spp. between influent and permeate inoculum passed through the (a) hollow fibre ultrafiltration, (b) spiral wound and (c) RO units.

5×10^5 cfu/mL influent to 5×10^4 cfu/mL permeate in the second and the third trials, respectively. This means that the rejection ranged 1-2 logs values.

Fig. 5b illustrates the efficiency of the spiral wound membrane in rejecting inoculated *Shigella* spp.. This membrane removed about 99.99% of the inoculated bacteria. The inoculated *Shigella* counts were 10^3, 10^4, 10^4 cfu/mL in the consecutive trials, and were reduced to 10^2, 10^2 and 10^1 cfu/mL respectively. The average rejection is 1-3 logs values, which demonstrates that the spiral wound membrane was efficient in terms of further removal of *Shigella* from the secondary treated wastewater without using chlorination or any method of disinfection.

Fig. 5c represents the terminal stage in the removal of *Shigella* spp. using RO system. The RO membrane showed a high efficiency in the removal of the residual bacteria. Although a count of 50-200 cfu/mL was passed through the RO membrane, the effluent of this membrane was free of *Shigella* spp..

4. Discussion

4.1 Microbial Removal after Activated Sludge Biological Treatment

The activated sludge secondary treatment (preliminary to the UF and RO processing) reduced the count of the screened bacterial parameters (TPC, TC and FC). This reduction is due to the known negative influence of the retention time on limiting the growth of bacteria as they compete for available nutrients and accumulate microbial wastes [24]. Chlorination of effluent coming from the activated sludge process completely eliminated the bacterial count in nearly all sampling dates (data are not shown). Chlorine affects bacteria by damaging their cells; it is considered as a non-selective oxidant negatively influencing the metabolic processes of microorganisms. Chlorine acts on microbial cell membrane altering its permeability, which leads to leakage of intracellular contents such as proteins and nucleic acids [25]. But chlorine concentration must be

finely tuned to the minimal inhibitory concentration (0.7 ppm) to avoid its negative effects on health and environment [26].

4.2 Microbial Removal after UF and RO Membranes

The hollow fibre membrane was inefficient to reduce total microbial count, total coliform, and faecal coliform bacteria received from unchlorinated activated sludge effluent. Therefore, the effluent generated by the hollow fibre membrane is not complying with the international standards concerning the recycling of wastewater for not restricted irrigation [6]. As mentioned before, chlorine was not used in our experiments because this oxidant can damage the RO system [27].

Results of the microbial indicators screened using the hollow fibre membranes (total plate count—TPC, total coliforms—TC, faecal coliforms—FC, *Escherichia coli*, *Enterococus* spp.) demonstrated that these microbes were not completely retained, despite the fact that the size of these bacteria ranges from 1 to 10 μm, and it is much larger than the size of the hollow fibre membrane pores, whose ranging is 0.005-0.04 μm [28]. The UF hollow fibre membrane having pores capable to remove 100 kDa molecules removed only 1 log of TPC (90% rejection, Table 3), but in some samples the reduction reached 1-2 logs of TC (90%-99% reduction, Table 4), and in other samples no rejection was observed. This means that the only use of this technology is ineffective to eliminate the microbial health hazards and must be coupled with a further treatment process if chlorination can not be adopted.

The spiral wound membrane was highly efficient to reject most of the total microbial count (99.9% rejection), and total coliform bacteria (99.99% rejection) in most sampling dates. It reduced TPC and TC approximately to zero in some cases. Furthermore, this membrane was able to completely eliminate FC in all trials. Therefore, also the subsequent RO effluents were free from TC and FC (Tables 4 and 5). The hollow fibre and spiral wound membranes reduced the microbial load of 2 and 4 logs values, respectively. Hereupon, their effluents to be passed through the RO membrane contained only low concentrations of these microbes, which were completely rejected in the last stage of the treatment (RO system). The whole filtration system provided an integral technology able to remove microbes from unchlorinated secondary treated wastewater.

Previous reports demonstrated that an integrated membrane system of MF (microfiltration) and UF has the potential to achieve a 7 logs reduction of pathogens [29]. Parker and co-workers [30] reported 3.3 and 4.3 logs reductions of TPC and TC, respectively, when using MF membrane having a pore size of 0.2 μm, while Willinghan and co-workers [31] reported a reduction of 2-6 logs values of TC and 4 logs values of FC. On the other hand, Kachalesky and co-workers [32] reported 1.8 and 2.2 log reduction of FC when UF membranes with 0.05 or 0.02 μm pore size were used, respectively. It was also reported that 1.2-1.7 logs reduction of TPC was achieved in treated wastewater and 2.1-2.2 log sreduction in surface water when they were passed through 0.03 μm UF membrane [33].

The hollow fibre UF system as a sole membrane filter was not efficient in rejecting *E. coli* and *Enterococus* spp. completely from water obtained with the activated sludge process. On the contrary, the spiral wound was significantly more efficient in reducing the number of these bacteria to undetectable count in its effluent used to feed the RO system, so that in this last stage the permeate was free of both bacteria.

Although the pores of the spiral wound membrane are much smaller than all waterborne bacterial species, a forward fouling of membranes due to a leak of bacteria through their pores was ascertained. Because the retention of bacteria having a size (1-10 μm) much larger than the absolute pore size of the MF and UF nominal pore diameters (0.005 to 0.05 μm) can not be

a problem as long as the filter is intact [34], the leakage of bacteria through membrane pores can be due to the existence of imperfections in the membrane structure that results in the presence of a number of defects such as pore doublets [22], little breakings of the fibre or tiny fractures of the seals [35].

On the other hand, the bacterial cell-wall structure is composed of a particular layer of a cross-linked polymer, the peptidoglycan, the thickness of which in Gram-negative bacteria is around 2 to 6 nm, whereas in Gram-positive bacteria it is thicker (around 20 to 80 nm). This thickness difference leads to different cell blow-out resistance, in the range of 0.3-0.5 and 25-35 bar for Gram-negative and Gram-positive bacteria, respectively [36]. The cross-linked polymer is responsible for the cell-wall mechanical strength and its elastic properties [37]. In addition, it can be assumed that the peptidoglycan cross-linkage characteristics may have an impact on the bacteria deformability depending on the nature and the number of existing transversal bridges [38]. Thus, the thinner this layer, the more the cell is deformable and likely can pass through smaller pores [39]. Gram-negative bacteria present a thin peptidoglycan layer, which allows their deformation and their passage through membrane pores smaller than their own size at rest ("deformable particle"). On the contrary, the large thickness of peptidoglycan layer in the Gram-positive bacteria limits their flexibility, which could prevent their transfer through smaller pores ("stiff particle").

E. coli is a Gram negative bacterium (elastic cell wall) having a diameter about 2 μm × 1 μm. It was inoculated in the hollow fibre membrane with a concentration of 10^6 cfu/mL, which was reduced of about 1-2 logs values (90%-99%) after filtration, while the same membrane was more efficient in removing *Enterococus* spp. (Gram positive) and reduced its count of 2-4 logs values (90%-99.99%). Further, the spiral wound membrane reduced *E. coli* (Gram negative) to further 3 logs (99.9%) compared to a reduction of *Enterococus* spp. (Gram positive) of about 5 logs (99.999%). *Enterococus* spp. is a bacterium having a hard cell wall, which preserves its cell shape and gives it resistance to be forced through pores smaller than the bacterial size. Gram negative bacteria *Salmonella* and *Shigella* were reduced after passing the hollow fibre membrane of around 1-2 logs values, which is in accordance with the results obtained with *E. coli*. Whereas spiral wound membrane was more efficient to reduce the bacterial load cutting off 4-5 logs for *Salmonella*, and 1-3 logs for *Shigella*. Naturally, the RO membrane with 0.001 μm pore size was the most efficient membrane to eliminate these microbes. The forward fouling of bacteria through membrane filters having smaller pore size that the bacterium size found in our results agrees with experimental data obtained in cross-flow filtration of *Pseudomonas* and *E. coli* species due to their cell wall structure as Gram negative bacteria [40-43].

Some studies proved that the spiral wound succeeded in the removal of viruses (0.005 to 0.1 μm), which are smaller than bacterial cells, while hollow fibre membrane did not remove viruses to any appreciable extent [29, 44]. Other researchers evaluated the effects of operating conditions on the filtration performance based on the behaviour and the viability of *E. coli* suspension under an exposure to an increased external osmotic pressure that caused a reduction of its cell volume by loosing part of the bacterial internal liquid (cytoplasm) [40, 41].

Concerning our experiment using the reverse osmosis system, it is important to underline that its membrane is capable to reject bacteria, salts, sugars, proteins, dyes and other constituents that have a molecular weight larger than 150-250 Dalton, but like all membranes, it is subjected to fouling by "cake layer" formation [22].

5. Conclusions

Integrated wastewater treatment plant including membrane separation proved to be very effective for

the elimination of pathogens from wastewater. Pathogen indicators such as total microbial count, total coliform as well as fecal coliform were found to be removed completely by sequential filtration using tight UF membrane (spiral wund UF) followed by reverse osmosis. On the other hand, loose UF membrane (hollow fibre UF) was found to be less efficient in removing these indicators. Modelling experiment using Grams negative bacteria such as *E. coli*, *Salmonella* and *Shigella* and Grams positive bacteria such as *Enterococus* spp. showed the same trend of efficiency with minute leakage through membrane pores even if they are smaller than the nominal size of the bacterial cells. This leakage was more pronounced for Gram negative bacteria compared to Gram positive ones because Gram negative bacteria have elastic cell walls with deformation ability when exposed to pressure, compared to that of Gram positive bacteria having stiff undeformable cell walls. These findings strongly pointed out the applicability of the use of a hybrid system of ultrafiltration and reverse osmosis technologies in sanitation of wastewater without the need of any invasive disinfectant that might lead to environmental and health hazards.

Acknowledgments

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References

[1] R. Al-Sa, Wastewater management for small communities in Palestine, Master Thesis, Birzeit University, Birzeit, Ramallah, West Bank, Palestine, 2000.

[2] Desk Study on the Environment in the Occupied Palestinian Territories [Online], United Nations International Program, UNEP, 2003, http://postconflict.unep.ch/publications/INF-31-WebOPT.pdf (accessed Oct. 12, 2011).

[3] G. Tchobanoglous, F.L. Burton, Wastewater Engineering: Treatment Disposal and Reuse, 3rd ed., McGraw-Hill Professional, Metcalf and Eddy Inc., New York, 1991.

[4] N. Mahmoud, Anaerobic pre-treatment of sewage under low temperature (15 ºC) conditions in an integrated UASB-digester system, Ph.D. Thesis, Wageningen University, Wageningen, The Netherlands, 2002.

[5] L. Seghezzo, Anaerobic treatment of domestic wastewater in subtropical regions, Ph.D. Thesis, Wageningen University, 2004.

[6] Health Guidelines for the Use of Wastewater Treatment and Health Safeguards, A report of WHO Meeting of Experts, Technical Report No. 517, WHO, Geneva, Switzerland, 1989.

[7] I. Hespanhol, Guidelines and integrated measures for public health protection in agricultural reuse systems, J. Water SRT-Aqua. 39 (1990) 227-249.

[8] I. Hespanhol, A.M.E. Post, WHO guidelines and national standards for reuse and water quality, Water Res. 28 (1994) 119-124.

[9] C.R. Jenkins, I. Papadopoulos, Y. Stylianous, Pathogens and wastewater use for irrigation in Cyprus, in: Proceeding of Land and Water Resources Management in the Mediterranean Region, Bari, Italy, 1994.

[10] T. Asano, A.D. Levine, Wastewater reclamation, recycling and reuse: Past, present and future, Water Sci. Tech. 33 (1996) 1-14.

[11] M.H. Marcos do Mont, A.N. Angelakis, T. Asano, Necessity and basis for establishment of European guidelines for reclimed wastewater in the Mediterranean region, Water Sci. Techol. 33 (1996) 303-316.

[12] M.A. Massoud, A. Tarhini, J.A. Nasr, Decentralized approaches to wastewater treatment and management: Applicability in developing countries, J. Environ. Manag. 90 (2009) 652-659.

[13] D. Barnes, P.J. Bliss, B.W. Gould, H.R. Valentine, Water and Wastewater Engineering Systems, Pitman Publishing Inc., Marshfield, Massachusetts, 1981.

[14] A.D. Levine, G. Tchobanoglous, T. Asano, Size distribution of particulate contaminants in wastewater and their impact on treatability, Water Res. 25 (1991) 911-922.

[15] R. Charles, Wastewater Management and Recovery, Lewis publishers, Boca Raton, Florida, USA, 1995.

[16] M.B. Pesccod, Wastewater Treatment and Use in Agriculture, FAO irrigation and drainage paper, No. 1.22-26.14.13, Rome, 1997, p. 47.

[17] J. Mark, J. Hammer, Water and Wastewater Technology, 4th ed., Prentice Hall Publisher, New Jersey Columbus, Ohio, 2001.

[18] S.R. Qasim, Wastewater Treatment Plants: Planning, Design and Operation, 2nd ed., Lancaster, Pennsylvania: Technomic, 1999.

[19] R.D. Sung, Effect of organic constituent in wastewater chlorination process, Ph.D. Thesis, University of California, Davis, CA, 1974.

[20] Copper in drinking water, Background Document for Development of WHO Guidelines for Drinking-Water Quality, 2004, World Health Organization Web site, http://www.who.int/water_sanitation_health/dwq/chemicals/copper.pdf (accessed Sept. 10, 2011).

[21] G. Belfort, Y. Rotem, E. Katzenelson, Virus concentration using hollow fibre membranes, Water Res. 9 (1974) 79-85.

[22] R. Radcliff, A. Zarnadze, Application of membrane technology to the production of drinking water, Water Conditioning and Purification 46 (2004) 23-25.

[23] Standard Methods for Examination of Water and Wastewater, 21st ed., American Water Work Association, Water Pollution Control Federation, Washington D.C., 2005.

[24] V. Kaimakamidou, T. Yiannakopoulou, Microbial food web and biosensors of waste water treatment process, in: Proceedings of the 12th International Symposium on Toxicity Assessment, Skiathos, Greece, 2005.

[25] C. Venkobachar, L. Iyengar, A.V.S.P. Rao, Mechanism of disinfection: Effect of chlorine on cell membrane functions, Water Res. 11 (1997) 727-729.

[26] R. Virto, D. Sanz, I.A. lvarez, S. Condon, J. Raso, Relationship between inactivation kinetics of a Listeria monocytogenes suspension by chlorine and its chlorine demand, J. of Applied Microbiol. 97 (2004) 1281-1288.

[27] J. Koo1, J. Lee, Y. Jung, S. Hong, S. Yoon, Chlorine resistant membrane and the mechanism of membrane degradation by chlorine, Water Environment Federation Web site, http://www.csmfilter.com/upload/csm/case/case2_2009729103826.pdf (accessed June 2011).

[28] C. Lubello, R. Gori, A.M. de Bernardinis, G. Simonelli, Ultrafiltration as tertiary treatment for industrial reuse, Water Sci. Technol. 3 (2003) 161-168.

[29] Low Pressure Membrane Filtration for Pathogen Removal: Application, Implementation, and Regulatory Issues, Office of Water, EPA 815-COL-001, WHO, 2001.

[30] D.Y. Parker, M.J. Leonard, P. Barber, G. Bonic, W. Jones, K.L. Leavell, Microfiltration treatment of filter backwash recycle water from a drinking water treatment facility, in: Proceedings of the AWWA Water Quality Technology Conference, American Water Works Association, Denver, 1999.

[31] G.A. Willinghan, J.C. Vickers, C. McGahey, Microfiltration as tertiary treatment eighteen month trial, in: Proceedings of the AWWA Membrane Technology Conference, Baltimore, MD, 1993.

[32] L.A. Kachalesky, T. Masterson, Membrane filtration of sewage treatment plant effluent, in: AWWA Membrane Technology Conference Proceedings, Reno, NV, 1995.

[33] Environmental technology verification report: Physical removal of Cryptosporidium oocysts and Giardia cysts in drinking water, Aquasource North America Ultrafiltration Membrane System Model A35, Pittsburgh, PA, 2000.

[34] A.J.M. Von Gottberg, J.M. Persechino, Using membrane filtration as pretreatment for reverse osmosis to improve system performance, in: North American Biennial Conference of the American Desalting Association, Ionics Technical Paper, 2000.

[35] S.S. Adham, J.G. Jacangelo, J.M. Laine, Low-pressure membranes: Assessing integrity, J. AWWA. 87 (1995) 3-62.

[36] S.B. Sadr Ghayeni, S.S. Madaeni, A.G. Fane, R.P. Schneider, Aspects of microfiltration and reverse osmosis in municipal wastewater reuse, Desalination 106 (1999) 25-29.

[37] X. Yao, M. Jericho, D. Pink, T. Beveridge, Thickness and elasticity of gram negative murein sacculi measured by atomic force microscopy, J. Bacteriol. 181 (1999) 6865-6875.

[38] A.V. Bolshakova, O.I. Kiselyova, I.V. Yaminsky, Microbial surfaces investigated using atomic force microscopy, Biotechnol. Prog. 20 (2004) 1615.

[39] N. Lebleu, C. Roques, P. Aimar, C. Causserand, Role of the cell-wall structure in the retention of bacteria by microfiltration membranes, J. Membrane Sci. 326 (2009) 178-185.

[40] Y. Mille, L. Beney, P. Gervais, Viabilty of *Escherichia coli* after combined osmotic and thermal treatment: A plasma membrane implication, Biochim. Biophys. Acta. 1567 (2002) 41-48.

[41] A.E. Smith, K.E. Moxham, A.P.J. Middelberg, On uniquely determining cell-wall material properties with compression experiment, Chem. Eng. Sci. 53 (1998) 3913-3922.

[42] W.M. Lu, K.L. Tung, S.M. Hung, K.J. Hwang, Compression of deformable gel particles, Powder Technol. 116 (2001) 1-12.

[43] K.J.Y. Hwang, M. Yu, Cross-flow microfiltration of submicron microbial suspension, J. Membrane. Sci. 194 (2001) 229-243.

[44] J.G. Jacangelo, S.S. Adham, J.M. Lainé, Mechanism of *Cryptosporidium*, *Giardia*, and MS2 virus removal by MF and UF, J. AWWA. 87 (1995) 107-121.