

Membrane Bioreactor Technology for Wastewater Reclamation

A.A. Alaneny¹, W.M. El-Senousy¹, A.Z. Al-Herrawy¹, M.M. Abo-Aly², F.A. El-Gohary^{1*}

¹National Research Centre, Water Pollution Research Department, 33 Bohouth St., P.O. Box 12622, Dokki, Giza, Egypt.

²Chemistry Departments, Faculty of Science, Ain Shams University, Cairo, Egypt.

RESPONDING to the water shortage in Egypt, the use of submerged membrane bioreactor (SMBR) for wastewater reclamation has been studied. The impact of hydraulic retention time (HRT), as the most influential operational parameter has been investigated. A bench-scale module was operated at three HRT: 6, 10 & 15h. The results demonstrated that total COD and BOD removal rates were not affected by changing the HRT or by variations in raw wastewater strength. Average residual COD values in the membrane permeate ranged from 14 to 20.3 mg O₂ L⁻¹. Corresponding residual BOD was below 3.0 mgL⁻¹. Nitrification capacity of the SMBR was high. HRT was found to be a key parameter for fouling. Decreasing the HRT enhances membrane fouling. At HRT of 6h, fouling happened after 6 days. Corresponding values for HRTs 10 and 15 were 13 day and more than 42 days, respectively. Total coliform bacteria counts in most of the MBR permeate samples were below the detection limit of 10 CFU/100 mL. All protozoan parasitic stages detected in raw wastewater were removed by the SMBR. At HRT of 15h, both human rotaviruses and adenoviruses genome copies were reduced by 2 to 3 log₁₀. This ratio was reduced by decreasing the HRT.

Keywords: Wastewater reclamation, Membrane bioreactor, Pathogens reduction, Membrane fouling.

Introduction

Water shortage in Egypt has created the demand to optimize the use of non-conventional water resources, the most important of which is municipal wastewater. However, pathogenic microorganisms present in human wastes are the primary hazard in water recycling applications schemes. As the population in Egypt continues to rise, there is a greater demand and increased pressure from the public to minimize the potential health risk involved in exposing the people to chemical and microbial contaminants. Pathogens most common in biologically treated secondary wastewater include the environmentally resistant cysts of *Giardia lamblia* and a variety of enteric bacteria and viruses. To ensure microbial safe water production, the secondary effluent is generally subjected to a further tertiary treatment by sand filtration [1], UV radiation [2]. Also, chemical disinfection using chlorine, ozone, and peracetic acid is frequently used [3-6]. However, the formation of harmful disinfection by-products

(e.g. THM) and the persistence of disinfection residues cause adverse health and environmental effects [3, 7]. Therefore, increased attention has been focused on the development of alternative techniques to the conventional activated sludge treatment. Recently, the implementation of submerged membrane bioreactors (SMBRs), as a non-hazardous advanced treatment alternative has attracted a great deal of attention from academia and decision makers in Egypt, especially where a high quality effluent is desired and plant footprint is constrained [8].

Submerged membrane bioreactor (SMBR) is a modification of the activated sludge process. It combines biological oxidation and membrane filtration into one unit process, and provide better and more consistent effluent quality than conventional activated sludge, regardless of influent quality. Microorganisms such as protozoa and bacteria, larger than the nominal pore sizes of micro membranes are expected to be removed completely [9], whereas viruses

*Corresponding author e-mail: fgohary@hotmail.com

Tel.: +201005272233

DOI: 10.21608/ejchem.2018.4128.1361

©2017 National Information and Documentation Center (NIDOC)

are not removed by filtration through micro membranes due to their smaller size. At the same time, the epidemiological significance of viruses as water borne pathogens and the large number of enteric viruses excreted in human feces, made understanding the mechanism of virus removal by MBR an issue of interest [10]. According to Chaudhry *et al.*, [11], virus removal by MBRs occurs via four mechanisms: (i) incorporation of viruses into the mixed liquor suspended solids (ii) retention by the clean backwashed membrane, (iii) retention by the cake layer formed on the membrane surface after a period of operation, and (iv) inactivation of the viruses within mixed liquor due to extracellular enzymes and predation. Previous studies showed that pathogenic bacteria and viruses are usually adsorbed onto the surfaces of suspended solids [12-14]. However, many factors are thought to affect the concentration of mixed liquor suspended solids and the formation of the cake layer. Thus, there is a need to obtain a better understanding of factors affecting chemical and microbiological contaminants removal to develop more effective design and operation plans for MBR applications. In general, HRT has a significant impact on biomass characteristics in the activated sludge system as the change in HRT alters the organic loading rate (OLR). The shorter the HRT, the higher is the OLR consequently the F/M ratio, which in turn affects sludge quantity and quality, dissolved oxygen (DO) concentration and MBR fouling. It also accelerates overgrowth of filamentous bacteria, along with the generation of larger, more irregular and porous sludge flocks [15]. In addition, it is directly related to reactor volume which affects the capital and operational costs [15]. For these reasons the effect of hydraulic retention time on the performance,

fouling behavior, properties of mixed liquor and removal of pathogenic organisms using a SMBR for treatment of real municipal wastewater was the focus of this study.

Material and Methods

Experimental set-up

Figure 1 shows the configuration of the SMBR system used in this study. It consists of a 10 L bioreactor with a submerged hollow fibre membrane module made of polyvinylidene difluoride (PVDF). It has an area of 0.2 m² and an effective pore size of 0.2 μm. The SMBRs was continuously fed with municipal wastewater from a near-by activated sludge wastewater treatment plant using a peristaltic pump. The reactor was seeded with returned activated sludge from the same wastewater treatment plant. The mixed liquor suspended solids (MLSS) concentrations were 3.0, 5.5 and 6.4 g/L corresponding to the HRTs 15, 10 & 6h. Aeration was carried out by injecting air bubbles from the bottom of the membrane tank. This allows scouring of the membrane and mixing of the contents of the reactor as well. The mode of membrane filtration was outside to inside. Permeate was pumped out using a peristaltic pump at a constant flux of 3, 4.5 and 4.8 L/m² h corresponding to HRT of 15, 10 and 6h. Pressure gauge was used to monitor the transmembrane pressure (TMP). At HRT of 6 and 10 h, the membrane module was back washed once a day with water for 30 minutes. At HRT of 15h, washing was not necessary up to 42 days (the duration of this experimental run). When the TMP reached 0.8 bar, the membrane was chemically cleaned using 0.5% sodium hypochlorite for 4 h followed by 1% citric acid for 1.5 h.

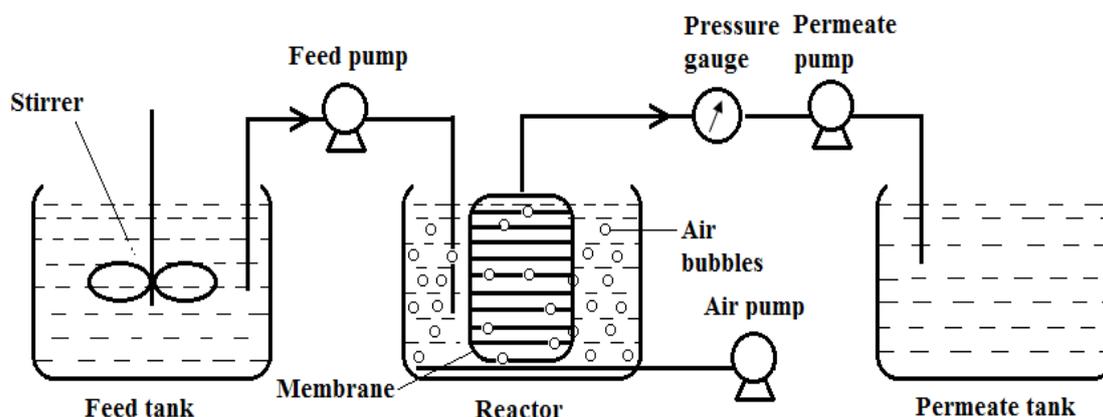


Fig. 1. Schematic diagram of the lab-scale SMBR treatment system.

Analytical procedures

The MBR performance was evaluated by monitoring the changes in the physical, chemical and microbiological characteristics of the influent and permeate (effluent), the membrane permeability and the sludge quality.

Physico-chemical analysis

Physical-chemical parameters, including chemical oxygen demand (COD), total nitrogen (TN), ammonia (NH₄-N), nitrite (NO₂-N), nitrate (NO₃-N), pH, dissolved oxygen (DO), were measured according to the standard methods for the examination of water and wastewater [16]. Once a week, total and volatile mixed liquor suspended solids were determined by filtering the mixed liquor through a 0.45 µm filter (GFC Whatman) paper.

Microbiological examination

Total and faecal coliform counts

Examination of total and faecal coliform counts was carried out using multiple tube fermentation technique [16].

Parasitological Examination

One liter wastewater samples (raw and SMBR effluent) were collected in autoclaved polypropylene plastic containers and subjected to parasitological examination at the same day of collection. Parasitological analysis was conducted using Bailenger [17] technique modified by Bouhoum and Schwartzbrod [18]. Samples were processed according to South Africa National Standard Method as follows: one liter wastewater samples (raw & treated effluent) were filtered through 150µm and 20µm filter papers. The suspended solid shield back by the 150µm filter were discarded, whilst those collected by the 20µm filter were rinsed off into a plastic beaker. The contents of the beaker were poured into test tubes and then centrifuged at 1389g for 3min. The obtained deposits were combined with zinc sulphate (ZnSO₄) floating solution at a specific gravity 1.3. The mixture was centrifuged at 617g for 3min. The obtained supernatant (about 1-2ml), containing the helminth ova and protozoan parasites, was diluted with 20-30ml distilled water and centrifuged at 964g for 3min. The final sediment was transferred to one or more microscope slides and microscopically examined to differentiate and enumerate parasitic helminth ova and comparatively large protozoan parasitic cyst/oocyst such as *Giardia*, *Entamoeba*, *Balantidium* and *Isospora* [19, 20]. After that, the previously examined slides were air dried

and fixed with absolute methyl alcohol. Fixed slides were stained with acid fast trichrome, mounted with DPX mounting solution, covered with glass slip and examined with oil immersion lens under the microscope for the detection of smaller organisms like *Cryptosporidium* spp., *Cyclosporacayetanensis* and microsporidial spores [21].

Virological Examination

a) Concentration of wastewater Samples

Influent and effluent wastewater samples (one liter) were concentrated by filtration through negatively charged nitrocellulose membranes (ALBET-Spain, 0.45 µm pore size, and 142 mm diameter filter series) after addition of AlCl₃ to a final concentration of 0.5 mM, acidification to pH 3.5 and after passing through Whatmann No. 1 filter paper. The viruses adsorbed to the membrane were eluted with 75 ml of 0.05 M glycine buffer, pH 9.5 (using HCl 5 N) containing 3 % beef extract (Lab-Limco powder, OXOID, UK) [22, 23]. Eluted viruses were re-concentrated by polyethylene glycol precipitation (PEG; Lewis and Metcalf [24]). Samples were neutralized and kept at -70 °C until used.

b) Viral Nucleic Acid Extraction

Viral RNA was extracted from 140 µl of the supernatant using BIOZOL Total RNA extraction reagent (BIOFLUX-Japan) and according to the manufacturer's instructions to a 30 µl final volume.

c) RT-PCR of a Fragment of the VP6-Coding Gene of Rotaviruses Group A

The primers used for RT-PCR were the forward VP6-F 5-GACGGNGCNACTACATGGT-3 and the reverse VP6-R 5-GTCCAATTCATNCCTGGTGG-3 primers (1 µm for each), and according to Gómara et al., [25] using 200 U of M-MLV reverse transcriptase enzyme (Promega-USA) in a total volume of 10 µl and 1.5 U of Taq DNA polymerase (Biobasic-Canada) in a total volume of 50 µl. Nested PCR amplification of the target rotavirus VP6 fragment was performed using the forward primer, VP6-NF 5-GCTAGAAATTTTGATACA-3, and the reverse primer, VP6-NR 5-TCTGCAGTTTGTGAATC-3 (1 µm for each), and according to Gallimore et al., [26] to amplify 155 bp fragment. PCR products (10 µl) were analyzed by electrophoresis on 3 % agarose gels (Panreac—Spain).

d) Quantification of Rotavirus Group A Genome Copies Using Real-Time RT-PCR Method

Real-time TaqMan RT-PCR was performed for positive samples in the previous RT-PCR screening. Real-time PCR was done using rotavirus@ceeramTools™ Food & Environmental kit and according to manufacturer's instructions using Rotavirus - Q Standard (Ceeram Tools), internal control, and Mengo Extraction Control (Ceeram Tools) and using a real-time PCR thermal cycler (Rotor-Gene Q, Qiagen). Raw rotavirus genome copy numbers measured by real-time RT-PCR, in duplicate, were corrected according to virus/nucleic acid extraction and RT-PCR efficiencies.

e) Cell Culture RT-PCR (CC-RT-PCR) for Quantification of Infectious Rotavirus Particles

Rotavirus CC-RT-PCR assay was performed according to Abad *et al.*, [27]; El-Senousy *et al.*, [28]; and El-Deeb Ghazy *et al.*, [29]. The assay was performed on suspensions of infected MA104 cells. Primers VP6-F and VP6-R were used. The RT-PCR method was the same as described previously. The detection limit in this tissue culture assay using 100 µl of inoculum is 1×10^1 CC-RT-PCR units/ml, where CC-RT-PCR unit is the reciprocal endpoint dilution detectable by CC-RT-PCR.

f) Extraction of DNA

It was done as described previously by Kapperud and co-workers [30] and modified by Lucero Estrada *et al.*, [31]. Fifty µl of sample concentrate were added to 50 µl of 1X PCR buffer containing 0.2mg of Proteinase K/ml. After being incubated at 37°C for 1h, the suspension was boiled for 10 min and then centrifuged at 12500 rpm for 5 min at 4°C. The supernatant was used for performing the PCR.

g) Detection of Adenoviruses Using Nested PCR

It was done according to Puig, M. *et al.*[32] using the specific primers hex AA 1885, hex AA 1913 for the first round PCR and nehex AA 1893 and nehex AA 1905 for the second round PCR for detection of human adenovirus and were selected from the DNA sequence of the open reading frame of hexon gene. PCR products (10 µl) were analyzed by electrophoresis on 3% agarose gels (Panreac- Spain).

h) Real-Time PCR for Quantification of Adenoviruses

Real-time TaqMan PCR was performed for positive samples in the previous PCR screening. Real-time PCR was done using adenovirus@

ceeram Tools™ Food & Environmental kit and according to manufacturer's instructions using adenovirus - Q Standard (Ceeram Tools), internal control, and Mengo Extraction Control (Ceeram Tools) and using a real-time PCR thermal cycler (Rotor-Gene Q, Qiagen). Raw adenovirus genome copy numbers measured by real-time RT-PCR, in duplicate, were corrected according to virus/nucleic acid extraction and RT-PCR efficiencies.

i) Cell Culture-PCR (CC-PCR) Technique for Quantification of Adenovirus Infectious Units

It was done according to Esawy *et al.*, [33] and Abdo *et al.*, [34] Adenovirus cell culture-PCR (CC-PCR) assay was performed on suspensions of the infected Hep-2 cell line. Set of primers, hex AA 1885 and hex AA 1913 was used. The detection limit in this tissue culture assay using 100 µl of inoculum is 1×10^1 CC-PCR units/ml (u/ml). An adenovirus CC-PCR unit is defined as the reciprocal endpoint dilution detectable by CC-PCR.

Calculations of membrane resistance fraction

The quantitative determination of the permeate flux (J) in L/ (h.m²) was calculated using Eq. 1:

$$J = Q/A_m \quad (1)$$

Where Q is the permeate flow rate (L/h) evaluated by measuring the collected effluent volume versus time, and A_m is the membrane surface area (m²).

The total membrane resistance was calculated according to Lee *et al.*, [35] using (Eq. 2)

$$J = \Delta P / \mu \cdot R_t \quad (2)$$

Where ΔP is the transmembrane pressure (N/m²), μ is the effluent viscosity (N.s/m²),

$$R_t = R_m + R_c + R_f \quad (3)$$

Where R_m is the initial membrane resistance, (R_f) the total organic and inorganic fouling resistance, (R_c) the sludge layer resistance coating membrane surface during filtration. R_m was determined by filtrating deionized water using the new membrane. In this case the sum of R_f and R_c equals zero and as a consequence R_t = R_m. The value of R_f was determined at the end of each run after removing the sludge layer.

Results and Discussion

Removal efficiency of SMBR for COD, BOD and SS

The lab-scale SMBR was fed continuously with wastewater from a full scale treatment plant;

therefore, influent COD concentration was not steady. During the study period, influent COD values to the SMBR system ranged from 250 to 475 mg O₂ L⁻¹. The COD values of treated wastewater by SMBR were 14.06 ±5.2, 19.91 ±2.4 and 20.3 ±4 for HRTs of 15, 10 and 6h, respectively. BOD followed the same pattern. It was below 3.0mg/L at the three HRT investigated. Available data (Table 1) indicates that the SMBR could ensure a very low and stable effluent COD, BOD and SS. It is however worth mentioning that at HRTs of 15, 10 and 6h, the average OLRs were 0.39, 0.45 and 0.72 kg BOD/m³.d, respectively. This is within the normal OLR range for activated sludge

systems. Corresponding food to microorganism (F/M) ratios were 0.136, 0.145 and 0.196 kg BOD₅/kg MLVSS d, respectively, which were also within the normal F/M range for activated sludge systems (0.2 to 0.5 kg BOD₅/kg MLVSS d). Hence, although the lower HRT increased OLR, COD and BOD, percentage removal values were not reduced significantly (Fig. 2). According to Meng et al. [36] COD removal is not affected by the HRT. But at the same time, the operation of the SMBR at low HRT lead to a reduction in dissolved oxygen concentration and hence in biomass activity in the mixed liquor suspended solids.

TABLE 1. Average values of influent and effluent wastewater and removal efficiency at different HRT.

HRT 6h			HRT 10h			HRT 15h			Unit	Parameters
R%	Effluent	Influent	R%	Effluent	Influent	R%	Effluent	Influent		
–	7.8±0.3	7.3±0.3	–	7.56±0.21	7.2±0.23	–	8.26±0.3	7.5±0.3	–	pH
100	0	195.2±36.4	100	0	221.8±51	100	0	177±51	mg/L	TSS (105 °C)
94.4	20.3±4	353.8±64.4	94.8	19.91±2.40	389±42	96.3	14.06±5.2	381±27.5	mg O ₂ /L	COD _{tot.}
98.6	2.6±0.8	188.5±21.3	98.7	2.96±1.32	223.7±37	98.7	2.58±1.2	213±66.8	mg O ₂ /L	BOD _{tot.}
94.4	1.2±0.55	22±3.1	92.8	1.54±1.97	21.4±2.8	100	0±0	16.4±4.1	mg N/L	Ammonia
–	1.02±0.5	0.06±0.1	–	1.26±1.93	0.04±0.07	–	0.67±0.4	0.04±0.03	mg N/L	Nitrite
–	29.1±3.8	0.83±0.4	–	29.45±3.39	0.85±0.65	–	25.86±9	0.8±0.8	mg N/L	Nitrate
89	3.7±1.9	35±3.5	91	3.67±5.82	39.3±5.7	94.5	1.42±0.9	26±8.8	mg N/L	TKN
31	1.1±0.2	1.60±0.2	20.6	2.81±1.7	3.54±2.2	25.2	1.42±0.9	1.9±0.9	mg P/L	TPO ₄

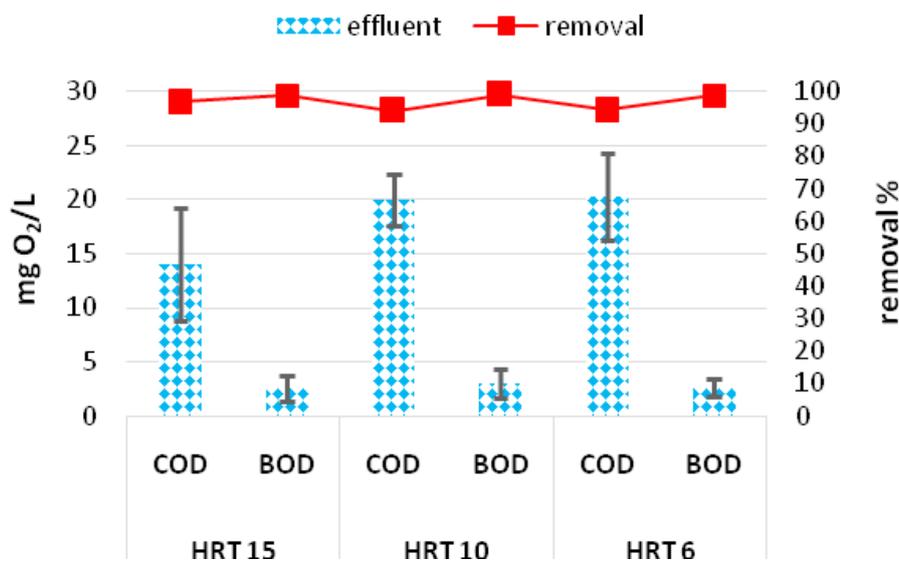


Fig. 2. Average values of residual COD and BOD in SMBR effluent.

Nitrogen removal

Table 1 summarizes the average concentrations of Ammonia (NH₄-N), Nitrite (NO₂-N), Nitrate (NO₃-N) and TKN. Complete removal of ammonia nitrogen has been reported at HRT of 15h. Corresponding values at HRTs of 10 and 6h were above 92%. There was no significant difference in the nitrification process between HRTs of 10 and 6h. These results agree with those previously reported by Liu *et al.*, [37]. According to Gander *et al.*, [38], the higher nitrification capacity of the MBR, as compared to conventional activated sludge is attributed to the higher sludge retention time (SRT). The smaller flock size in the high sludge age MBR helps microorganisms be exposed to oxygen and nutrients much more easily.

Total and fecal coliform bacteria removal

Results of bacteriological examination showed that influent total coliform bacteria count ranged from 7.5x 10⁶ CFU/100 mL to 4.7x 10⁷ CFU/100 mL with a median value of 2.16x10⁷ colony forming unit/100 mL (CFU/100 mL). Corresponding values of fecal coli form bacteria in the influent ranged from 4.8 x10⁵ to 2.8x10⁶ CFU/100 mL with a median value of 1.85x10⁶CFU/100 mL. Since the size of coliform bacteria ranges from 0.6 to 1.2 mm in diameter and from 2 to 3 mm in length [39], which is larger than the pore size of the membrane used in the present study (0.2um), the total coliform bacteria count in most of the MBR permeate examined samples were below the detection limit of 10 CFU/100 mL. Few samples contained higher counts which could be due to contamination during backwash of the filtrate line. Similar observations have been mentioned by Zhang, K. *et al.* [40] and Adham, S.

et al. [41].

Parasites removal

Parasitic helminth ova were detected in 33.3% of the raw wastewater samples investigated and disappeared completely from the treated wastewater. Parasitic helminth ova of nematodes and cestodes were detected in 25 and 8.3% of the examined raw wastewater samples, respectively, but no parasitic trematode ova were detected (Fig. 3).

On the other hand, parasitic protozoa were detected in most of the examined raw wastewater samples (91.7%) and disappeared in treated wastewater, followed by apicomplexan oocysts (16.7%) and sarcodine cysts (8.3%), but no parasitic flagellates and parasitic ciliates were detected. All the detected protozoan parasitic stages in raw wastewater were removed by treatment using the SMBR (Fig. 3).

Genera of intestinal helminths, *Ascaris* spp. ova and *Trichostrongylus* spp. Ova, related to nematode helminths were detected in only in raw wastewater samples in percentages 16.7 and 8.3%, respectively. These types of nematode ova were completely removed by treatment. *Hymenolepis* ova were the only cestode ova that occurred in 8.3% of raw wastewater samples and they were not detected in the effluent. No trematode ova were detected in raw or treated wastewater (Fig. 4).

Cysts of *Entamoeba* spp. and oocysts of *Cryptosporidium* spp. were found in the examined raw wastewater samples at 8.3 and 16.7, respectively. After treatment it disappeared completely.

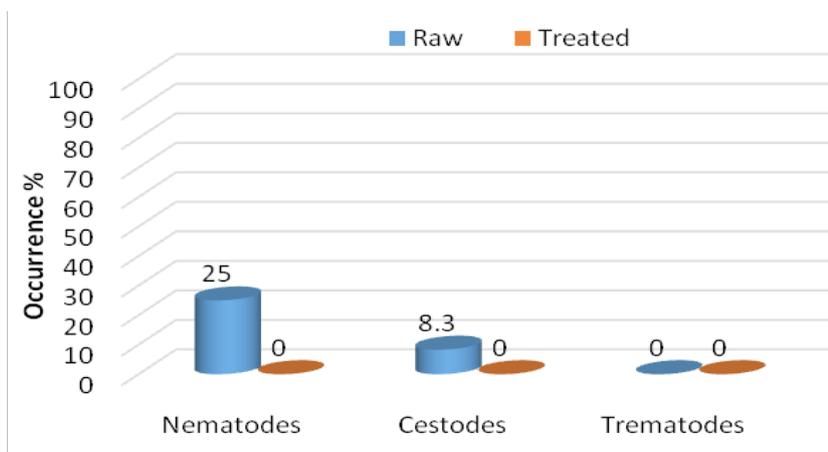


Fig. 3. Occurrence of helminth eggs in raw and treated wastewater.

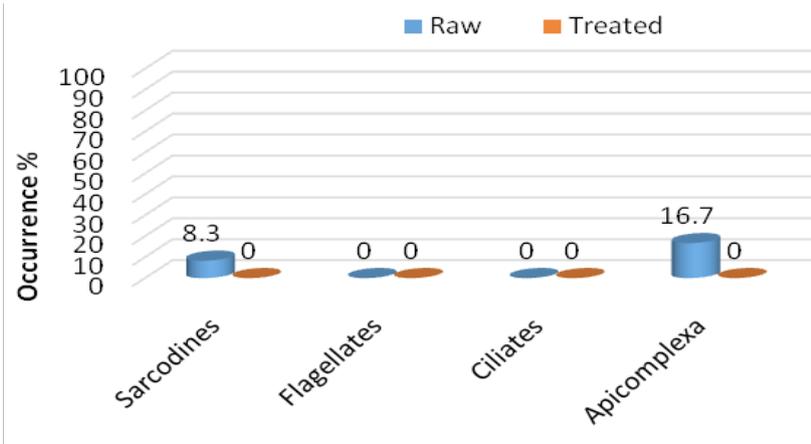


Fig. 4. Occurrence of parasitic protozoa in raw and treated wastewater.

Virus Removal

In the present study, the use of mengo virus as concentration/extraction processes control for both rotaviruses and adenoviruses showed mean processes efficiency for rotaviruses and adenoviruses in raw sewage samples of $10.8\% \pm 0.56$ and $11.9\% \pm 0.60$, respectively. The efficiency was increased to $17.1\% \pm 0.63$ and $18.1\% \pm 0.78$ for rotaviruses and adenoviruses in treated samples (Fig. 5). On the other hand, the mean efficiency of real time RT-PCR of rotaviruses for both raw and treated samples were $86.9\% \pm 2.49$ and $89.5\% \pm 2.47$ respectively. In case of adenoviruses, the mean efficiency of real time PCR increased to $88.2\% \pm 2.22$ and $90.3\% \pm 2.29$ for raw and treated samples respectively (Fig. 6). The correction of results according to the efficiencies of either concentration/extraction or real time RT-PCR/PCR processes may affect directly the final results of both raw and treated samples. The higher accuracy of results according to the use of mengo virus as concentration/extraction process control and internal control for rotaviruses and the other internal control for adenoviruses corrects the results shown by the real time PCR machine as explained in different reports [42-44]. This correction which gives the accurate genome copy numbers in both raw sewage and treated samples gives accurate efficiencies of the treatment processes. The difference of concentration/extraction processes efficiency in addition to the efficiency of the real time RT-PCR/PCR processes between raw sewage and treated effluents according to the difference in inhibitors in both kinds of samples as shown in our results may change the difference in the final genome copy numbers in the raw and

treated samples and consequently the efficiency of the treatment processes. El-Senousy and co-workers [42] reported an efficiency mean of concentration/extraction processes using mengo virus $92.2\% \pm 11.7$ in irrigation water samples. In the same study the real time RT-PCR process efficiency mean was $93.2\% \pm 4.8$. The difference in the results especially in case of concentration/extraction processes efficiency may return to the type of samples, time and region of sampling. All these factors affect the quantity and type of inhibitors in the tested samples. The accurate estimation of the efficiency of the different wastewater treatment processes is very important in the field of public health however there is an increasing importance of using the treated sewage in irrigation of different kinds of crops depending on the efficiency of the treatment processes.

In our study, results of the virus examination revealed the presence of a direct relationship between the number of genome copies and the number of infectious units of both human rotaviruses and adenoviruses in the raw wastewater with 2 to 3 log higher numbers of genome copies than infectious units for both rotaviruses and adenoviruses. This is usually attributed to the higher survival rate of genome than the entire viral particles in the environment [27, 45]. Since the system was fed with natural sewage, the presence of viruses in the feed was affected by the seasonal changes. This was clear in the absence of rotaviruses in the raw wastewater during spring and summer seasons. The peak of rotaviruses is in autumn and winter [46, 47]. Adenoviruses which have no seasonal variations were detected all the year around [48-53].

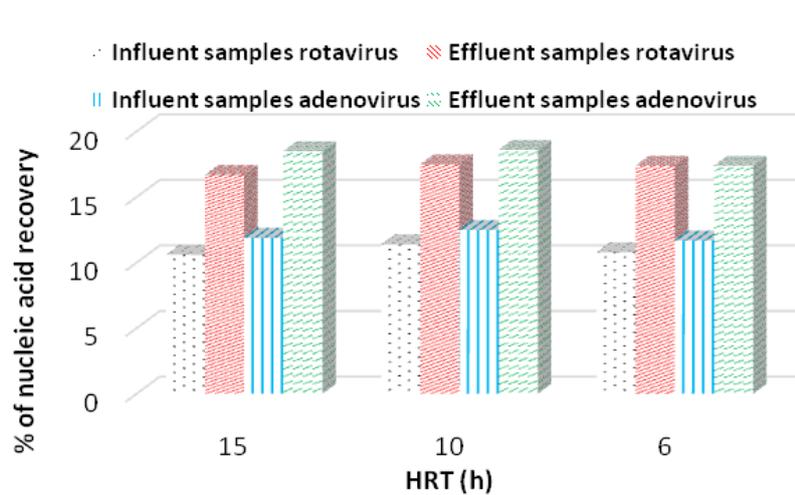


Fig. 5. Percentage of nucleic acid recovery efficiency of rotavirus and adenovirus in both influent and effluent samples at different HRT

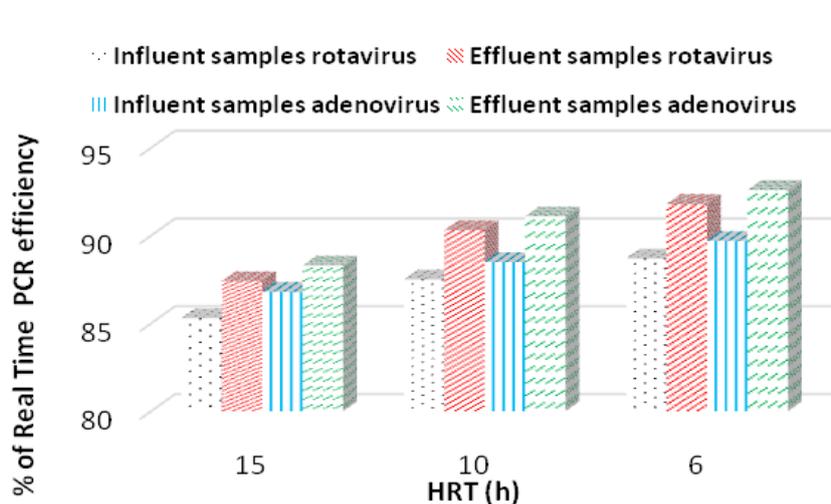


Fig. 6. Percentage of Real Time PCR efficiency of rotavirus and adenovirus in both influent and effluent samples at different HRT.

The reductions of both human rotavirus and adenovirus genome copies and their infectious units, at different HRTs are presented in Fig. 7. Results show that at HRT of 15h, removal of both human rotaviruses and adenoviruses genome copies ranged from 2 to 3 \log_{10} with a mean value of 2.8 \log_{10} and 2.3 \log_{10} , respectively. At the same time, the highest removal value for the infectious units of both rotaviruses and adenoviruses was 2 \log_{10} . At HRT of 10 and 6, no removal for adenoviruses infectious units has been reported. In general, the reduction of adenoviruses genome copies in our study is less than the reduction ratio of adenovirus genome copies (5.5 \log_{10}) reported by Simmons and co-workers [54] in a full-scale membrane bioreactor with nominal pore size 0.04 μm . In another study, removal efficiency of

the viral genome in the full-scale MBR process (MBR; nominal pore size 0.04 μm) was assessed and showed an average human adenoviruses removal of 5 logs over the study period [55]. Also, modified MBR (The nominal molecular weight cutoff (MWCO) of this polyethersulfone (PES) membrane is 150 kDa) using grafted zwitterionic polymer hydrogels achieved 5 \log_{10} removal of viral particles of human adenoviruses type 2 [56]. This could be explained as a result of the relatively large pore size of the membrane used in this study. Taking into consideration the size of Rotaviruses (65-70 nm) [57] and that of adenoviruses (90-100 nm)[58], it can be concluded that in our study where MBR with pore size 0.2 μm is used, virus removal cannot depend on filtration, but on other different mechanism such as: (i) attachment of

virus to mixed liquor solids; (ii) virus retention by a just backwashed membrane; (iii) virus

retention by the membrane cake layer; and/or (iv) inactivation.

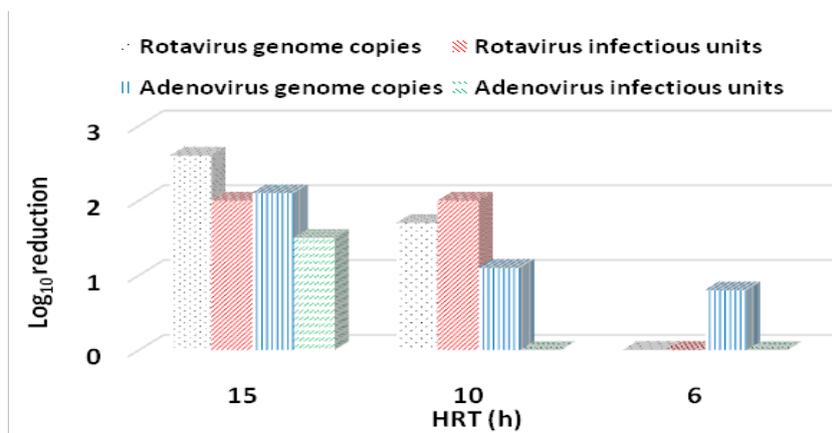


Fig. 7. Log₁₀ reduction of genome copies and infectious units of rotaviruses and adenoviruses at different HRT.

Biomass characteristics

The biomass concentrations in the SMBR fluctuated between 3950 and 5150 mg/L. At HRTs of 6, 10 and 15 h, the average MLSS concentrations were 5150, 4950 and 3950 mg/l, respectively. Moreover, limited biomass production resulting from the operation of the MBR at low F/M ratio was compensated by sludge withdrawn for analysis. The average MLVSS/MLSS ratio ranged from 0.73 to 0.83.

Fouling behaviour

Membrane filtration performance

To investigate the fouling behavior of the

membrane, the change in TMP was monitored at the three HRTs 6, 10 and 15h corresponding to flux values of 4.6, 4.5 and 3. The typical TMP variations for the three runs are shown in Fig. 8. Available data indicate that membrane fouling can be reduced by operating the SMBR at low permeate flux (or longer HRT). Fouling period was found to be 6 days at HRT of 6h. Corresponding values for HRTs 10 and 15 were 13 and more than 42 days at HRT of 15h. These results confirm the findings of Chae et al. and Huang et al. who found that, at constant membrane surface area, operating the MBR at lower HRT enhances the membrane fouling [59, 60].

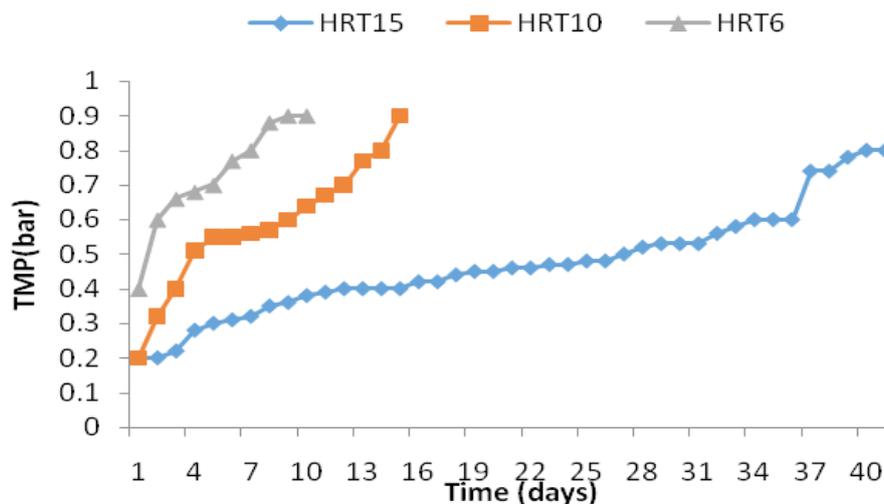


Fig. 8. Transmembrane profiles at different HRT.

Resistance analysis

To evaluate the membrane filtration characteristics, the different resistance values were calculated (Fig. 9). The total hydraulic resistance (R_t) was found to increase with increasing the flux rate. The irreversible fouling resistance

(R_f) fraction was almost the same at HRT 10 and 15. The cake layer resistance followed the same pattern and at the same time it is the major contributor towards membrane fouling. These results are in agreement with those reported by Khan, S.J. et al. [61].

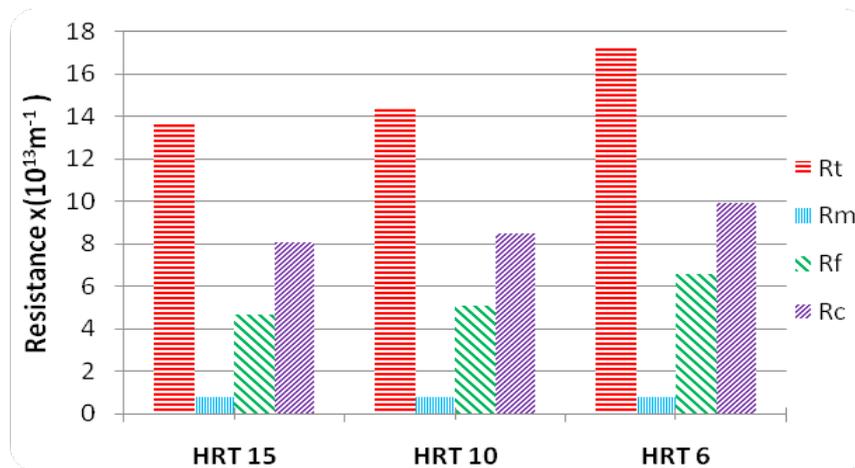


Fig. 9. Membrane resistance values at different HRT.

Conclusions

- Assessment of the results of this study indicated that SMBR can achieve efficient removals of suspended solids, organic contaminants parasites and fecal coliform bacteria. Enteric viruses were reduced by approximately 2Log_{10} . Since viruses cannot be removed by size exclusion, the reduction can be attributed to aggregation and adsorption to activated sludge or to the cake layer formed over the membrane.
- Membrane bioreactors MBRs can decrease the surface area needs for new wastewater treatment plants (WWTPs), and increases the treatment capacities of existing WWTPs at a given surface area.
- HRT is an important operational parameter which affects treatment performance, biomass characteristics, membrane fouling and pathogens removal.
- Operation at longer HRT (or low permeate flux) reduced the membrane fouling.
- A rapid rise in TMP resulted from short HRT.
- The filtration flux test, indicated that sludge cake formation was the main cause of the fouling process.

Acknowledgment

The authors acknowledge funding of this study from the National Research Centre, Egypt.

References

1. Zanetti, F., De Luca, G. and Sacchetti, R., Microbe removal in secondary effluent by filtration. *Ann. Microbiol.***56**, 313–317(2006).
2. Taghipour, F., Ultraviolet and ionizing radiation for microorganism inactivation. *Water Res.***38**, 3940–3948 (2004).
3. Chen, K.-C. and Wang, Y.-H., Control of disinfection by-product formation using ozone-based advanced oxidation processes. *Environ. Technol.***33**, 487–495(2012).
4. De Luca, G., Sacchetti, R., Zanetti, F. and Leoni, E., Comparative study on the efficiency of peracetic acid and chlorine dioxide at low doses in the disinfection of urban wastewaters. *Ann. Agric. Environ. Med.***15**, 217–224 (2008).
5. Koivunen, J. and Heinonen-Tanski, H., Peracetic acid (PAA) disinfection of primary, secondary and tertiary treated municipal wastewaters. *Water Res.***39**, 4445–4453 (2005).
6. Zanetti, F., De Luca, G., Sacchetti, R. and Stampi, S., Disinfection efficiency of peracetic acid (PAA):

- Inactivation of coliphages and bacterial indicators in a municipal wastewater plant. *Environ. Technol.* **28**, 1265–1271 (2007).
7. Wert, E.C., Rosario-Ortiz, F.L., Drury, D.D. and Snyder, S.A., Formation of oxidation byproducts from ozonation of wastewater. *Water Res.* **41**, 1481–1490(2007).
 8. El-Gohary, E.F., Integration of Wastewater in the Water Resources of Egypt: Opportunities and Constrains (2013).
 9. Ottoson, J., Hansen, A., Björleinius, B., Norder, H. and Stenström, T.A., Removal of viruses, parasitic protozoa and microbial indicators in conventional and membrane processes in a wastewater pilot plant. *Water Res.* **40**, 1449–1457 (2006).
 10. Simmons, F.J. and Xagorarakis, I., Release of infectious human enteric viruses by full-scale wastewater utilities. *Water Res.* **45**, 3590–3598 (2011).
 11. Chaudhry, R.M., Holloway, R.W., Cath, T.Y. and Nelson, K.L., Impact of virus surface characteristics on removal mechanisms within membrane bioreactors. *Water Res.* **84**, 144–152(2015).
 12. Chitnis, V., Chitnis, S., Vaidya, K., Ravikant, S., Patil, S. and Chitnis, D.S., Bacterial population changes in hospital effluent treatment plant in central India. *Water Res.* **38**, 441–447(2004).
 13. Otaki, M., Yano, K. and Ohgaki, S., Virus removal in a membrane separation process, in: *Water Science and Technology*. p. 107–116 (1998).
 14. Sakoda, A., Sakai, Y., Hayakawa, K. and Suzuki, M., Adsorption of viruses in water environment onto solid surfaces. *Water Sci. Technol.* **35**, 107–114 (1997).
 15. Hemmati, A., Dolatabad, M.M., Naeimpoor, F., Pak, A. and Mohammadi, T., Effect of hydraulic retention time and temperature on submerged membrane bioreactor (SMBR) performance. *Korean J. Chem. Eng.* **29**, 369–376(2012).
 16. American Public Health Association (APHA), Standard Methods for the Examination of Water and Wastewater. Stand. Methods, Washington, DC, USA, 541(2005).
 17. Bailenger, J., Valeur comparée des méthodes d'enrichissement en coprologie parasitaire. *Pharm. Biol.* **3**, 249–259(1962).
 18. Bouhoum, K. and Schwartzbrod, J., Quantification of helminth eggs in waste water. *Zentralblatt für Hyg. und Umweltmedizin. Int. J. Hyg. Environ. Med.* **188**, 322–30(1989).
 19. UK Standards for Microbiology Investigations, Staining Procedures. National Health Service, Standards Unit, Microbiology Services, PHE. Bacteriology – Test Procedures | TP 39 | **2.1**, 1-52(2015).
 20. Water Research Commission: Standard methods for the recovery and enumeration of helminth ova in wastewater, sludge, compost and urine–Diversion Waste in South Africa. WRC Report No. TT322/08(2008).
 21. Ignatius, R., Eisenblätter, M., Regnath, T., Mansmann, U., Futh, U., Hahn, H. and Wagner, J., Efficacy of different methods for detection of low *Cryptosporidium parvum* oocyst numbers or antigen concentrations in stool specimens. *Eur. J. Clin. Microbiol. Infect. Dis.* **16**, 732–736(1997).
 22. Rose, J.B., Singh, S.N., Gerba, C.P. and Kelley, L.M., Comparison of microporous filters for concentration of viruses from wastewater. *Appl. Environ. Microbiol.* **47**, 989–992 (1984).
 23. Smith, E.M. and Gerba, C.P., Development of a method for detection of human rotavirus in water and sewage. *Appl. Environ. Microbiol.* **43**, 1440–50 (1982).
 24. Lewis, Gillian D. and Theodore G. Metcalf., Polyethylene glycol precipitation for recovery of pathogenic viruses, including hepatitis A virus and human rotavirus, from oyster, water, and sediment samples. *Appl. Environ. Microbiol.* **54**, 1983–1988 (1988):.
 25. Gómara, M.I., Wong, C., Blome, S., Desselberger, U. and Gray, J., Molecular characterization of VP6 genes of human rotavirus isolates: correlation of genogroups with subgroups and evidence of independent segregation. *J. Virol.* **76**, 6596–6601 (2002).
 26. Gallimore, C.I., Taylor, C., Gennery, A.R., Cant, A.J., Galloway, A., Iturriza-Gomara, M. and Gray, J.J., Environmental monitoring for gastroenteric viruses in a pediatric primary immunodeficiency unit. *J. Clin. Microbiol.* **44**, 395–399 (2006).
 27. Abad, F.X., Pintó, R.M., Villena, C., Gajardo, R. and Bosch, A., Astrovirus survival in drinking water. *Appl. Environ. Microbiol.* **63**, 3119–3122 (1997).

28. El-Senousy, W.M., Guix, S., Abid, I., Pintó, R.M. and Bosch, A., Removal of astrovirus from water and sewage treatment plants, evaluated by a competitive reverse transcription-PCR. *Appl. Environ. Microbiol.* **73**, 164–167 (2007).
29. El-Deeb Ghazy, M.M., El-Senousy, W.M., Abdel-Aatty, A.M. and Kamel, M., Performance evaluation of a waste stabilization pond in a rural area in Egypt. *Am. J. Environ. Sci.* **4**, 316–326 (2008).
30. Kapperud, G., Vardnund, T., Skjerve, E., Hornes, E. and Michaelsen, T.E., Detection of pathogenic *Yersinia enterocolitica* in foods and water by immunomagnetic separation, nested polymerase chain reaction and colorimetric detection of amplified DNA. *Appl. Environ. Microbiol.* **59**, 2938–2944 (1993).
31. Lucero Estrada, C.S.M., Carmen Velázquez, L. del, Genaro, S. Di and Stefanini de Guzmán, A.M., Comparison of DNA extraction methods for pathogenic *Yersinia enterocolitica* detection from meat food by nested PCR. *Food Res. Int.* **40**, 637–642 (2007).
32. Puig, M., Jofre, J., Lucena, F., Allard, A., Wadell, G. and Girones, R., Detection of adenoviruses and enteroviruses in polluted waters by nested PCR amplification. *Appl. Environ. Microbiol.* **60**, 2963–2970 (1994).
33. Esawy, M.A., Ahmed, E.F., Helmy, W.A., Mansour, N.M., El-Senousy, W.M. and El-Safty, M.M., Production of levansucrase from novel honey *Bacillus subtilis* isolates capable of producing antiviral levans. *Carbohydr. Polym.* **86**, 823–830 (2011).
34. Abdo, S.M., El-Senousy, W.M., Ali, G.H., Hetta, M.H., Salah, R.A., Din, E. and Correspondence, F., Antiviral Activity of Freshwater Algae. *J. Appl. Pharm. Sci.* **02**, 21–25 (2012).
35. Lee, E.J., An, A.K.J., Hadi, P. and Yan, D.Y.S., Characterizing flat sheet membrane resistance fraction of chemically enhanced backflush. *Chem. Eng. J.* **284**, 61–67 (2016).
36. Meng, F., Shi, B., Yang, F. and Zhang, H., Effect of hydraulic retention time on membrane fouling and biomass characteristics in submerged membrane bioreactors. *Bioprocess Biosyst. Eng.* **30**, 359–367 (2007).
37. Liu, Q., Wang, X.C., Liu, Y., Yuan, H. and Du, Y., Performance of a hybrid membrane bioreactor in municipal wastewater treatment. *Desalination* **258**, 143–147 (2010).
38. Gander, M., Jefferson, B. and Judd, S., Aerobic MBRs for domestic wastewater treatment: A review with cost considerations. *Sep. Purif. Technol.* **18**, 119–130 (2000).
39. Hirani, Z.M., DeCarolis, J.F., Adham, S.S. and Jacangelo, J.G., Peak flux performance and microbial removal by selected membrane bioreactor systems. *Water Res.* **44**, 2431–2440 (2010).
40. Zhang, K. and Farahbakhsh, K., Removal of native coliphages and coliform bacteria from municipal wastewater by various wastewater treatment processes: Implications to water reuse. *Water Res.* **41**, 2816–2824 (2007).
41. Adham, S. and Decarolis, J.F., Optimization of Various MBR Systems for Water Reclamation: Phase III (2004).
42. El-Senousy, W.M., Costafreda, M.I., Pintó, R.M. and Bosch, A., Method validation for norovirus detection in naturally contaminated irrigation water and fresh produce. *Int. J. Food Microbiol.* **167**, 74–79 (2013).
43. Pintó, R.M., Costafreda, M.I. and Bosch, A., Risk assessment in shellfish-borne outbreaks of hepatitis A. *Appl. Environ. Microbiol.* **75**, 7350–7355 (2009).
44. Costafreda, M.I., Bosch, A. and Pintó, R.M., Development, evaluation, and standardization of a real-time TaqMan reverse transcription-PCR assay for quantification of hepatitis A virus in clinical and shellfish samples. *Appl. Environ. Microbiol.* **72**(6), 3846–3855 (2006).
45. El-Senousy, W.M. and Abou-Elela, S.I., Assessment and Evaluation of an Integrated Hybrid Anaerobic–Aerobic Sewage Treatment System for the Removal of Enteric Viruses. *Food Environ. Virol.* **9**, 287–303 (2017).
46. El-Senousy, W., Pintó, R. and Bosch, A., Epidemiology of human enteric viruses in the Cairo water environment, in: *1st International Conference of Environmental Research Division on Sustainable Development Environmental Challenges Facing Egypt. National Research Centre, Cairo, Egypt* (2004).
47. Villena, C., El-Senousy, W.M., Abad, F.X., Pintó, R.M. and Bosch, A., Group A rotavirus in sewage samples from Barcelona and Cairo: Emergence of

- unusual genotypes. *Appl. Environ. Microbiol.* **69**, 3919–3923 (2003).
48. Fong, T.T., Phanikumar, M.S., Xagorarakis, I. and Rose, J.B., Quantitative detection of human adenoviruses in wastewater and combined sewer overflows influencing a Michigan river. *Appl. Environ. Microbiol.* **76**, 715–723 (2010).
49. Griffin, D.W., Lipp, E.K., McLaughlin, M.R. and Rose, J.B., Marine Recreation and Public Health Microbiology: Quest for the Ideal Indicator. *Bioscience* **51**, 817 (2001).
50. Hundesa, A., Maluquer De Motes, C., Bofill-Mas, S., Albinana-Gimenez, N. and Girones, R., Identification of human and animal adenoviruses and polyomaviruses for determination of sources of fecal contamination in the environment. *Appl. Environ. Microbiol.* **72**, 7886–7893 (2006).
51. Jiang, S., Dezfulian, H. and Chu, W., Real-time quantitative PCR for enteric adenovirus serotype 40 in environmental waters. *Can. J. Microbiol.* **51**, 393–398 (2005).
52. Katayama, H., Haramoto, E., Oguma, K., Yamashita, H., Tajima, A., Nakajima, H. and Ohgaki, S., One-year monthly quantitative survey of noroviruses, enteroviruses, and adenoviruses in wastewater collected from six plants in Japan. *Water Res.* **42**, 1441–1448 (2008).
53. Pina, S., Puig, M., Lucena, F., Jofre, J. and Girones, R., Viral pollution in the environment and in shellfish: Human adenovirus detection by PCR as an index of human viruses. *Appl. Environ. Microbiol.* **64**, 3376–3382 (1998).
54. Simmons, F.J., Kuo, D.H.W. and Xagorarakis, I., Removal of human enteric viruses by a full-scale membrane bioreactor during municipal wastewater processing. *Water Res.* **45**, 2739–2750 (2011).
55. Kuo, D. H. W., Simmons, F. J., Blair, S., Hart, E., Rose, J. B. and Xagorarakis, I., Assessment of human adenovirus removal in a full-scale membrane bioreactor treating municipal wastewater. *Water Res.* **44**, 1520–1530 (2010).
56. Lu, R., Zhang, C., Piatkovsky, M., Ulbricht, M., Herzberg, M. and Nguyen, T.H., Improvement of virus removal using ultrafiltration membranes modified with grafted zwitterionic polymer hydrogels. *Water Res.* **116**, 86–94 (2017).
57. Kapikian, A.Z., Hoshino, Y. and Chanock, R.M., Rotaviruses. In: *Fields Virology*, 4th ed. by Knipe, D.M., Howley, P.M. Philadelphia: Lippincott, Williams and Wilkins, pp. 1787–1833 (2001).
58. Rowe, W.P., Huebner, R.J., Gilmore, L.K., Parrott, R.H. and Ward, T.G., Isolation of a Cytopathogenic Agent from Human Adenoids Undergoing Spontaneous Degeneration in Tissue Culture. *Exp. Biol. Med.* **84**, 570–573 (1953).
59. Chae, S.R., Ahn, Y.T., Kang, S.T. and Shin, H.S., Mitigated membrane fouling in a vertical submerged membrane bioreactor (VSMBR). *J. Memb. Sci.* **280**, 572–581 (2006).
60. Huang, Z., Ong, S.L. and Ng, H.Y., Submerged anaerobic membrane bioreactor for low-strength wastewater treatment: Effect of HRT and SRT on treatment performance and membrane fouling. *Water Res.* **45**, 705–713 (2011).
61. Khan, S.J., Ilyas, S. and Zohaib-Ur-Rehman, Impact of nitrogen loading rates on treatment performance of domestic wastewater and fouling propensity in submerged membrane bioreactor (MBR). *Bioresour. Technol.* **141**, 46–49 (2013).

(Received 11/6/2018;
accepted 11/7/2018)

استخدام تكنولوجيا المفاعل الحيوي الغشائي لمعالجة مياه الصرف الصحي لإعادة استخدامها

على السيد العيني^١، وليد مرسى السنوسى^١، احمد زكريا الهراوى^١، محمد محمود ابو على^٢، فاطمه عبد الحميد الجوهري^١

^١ قسم تلوث المياه - المركز القومى للبحوث - الجيزة - مصر.

^٢ كلية العلوم - جامعه عين شمس - القاهرة- مصر .

تواجه مصر حاليا عجز في مواردها المائيه العذبه نتيجة الزيادة السكانيه المطردة والتنمية الاقتصادية المتسارعه. والقطاع الزراعي هو المستخدم الأكبر للمياه في مصر. وتمثل حصته حوالي ٨٠٪ من المجموع الكلي لكل الاحتياجات ، ولمواجه هذا التحدي لجأت الحكومة المصرية الى سياسة زيادة مواردها المائية بموارد مائيه غير تقليديه مثل المياه العادمة المعالجة (المنزليه والصناعيه) وللحصول على مياه معالجه ليس لها تأثير سلبي على صحه المواطنين والبيئه ، كان لا بد من استخدام تكنولوجيا متقدمه مثل نظم الاغشيه المختلفه. لذلك تم اجراء هذه الدراسه باستخدام المفاعل الحيوي الغشائي المغمور (Hollow fiber) وذلك لاستنباط افضل اسس التصميم ومعايير التشغيل ومن اهمها الوقت المناسب لبقاء المياه في احواض التهويه (HRT) وكذلك حجم الحمأة واثر ذلك على نوعيه المياه المعالجه وانسداد الاغشيه . ولقد اوضحت النتائج المتحصل عليها ان تغيير زمن المكث من ٦ الى ١٥ ساعه لم يؤثر على نوعيه المياه المنتجه، غير ان تأثيره كان واضحا على انسداد الاغشيه المستخدمه . كما اوضحت النتائج امكانية التخلص الكامل من البكتريا القولونيه وكذلك الطفيليات. اما بالنسبه للفيروسات. فنظرا لصغر حجمها عن فتحات المرشح الغشائي فلقد توقفت نسبه ازاله على ادمصاصها على الحمأة داخل المفاعل .