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Removal of indicator bacteriophages from municipal wastewater by a full-scale membrane bioreactor and a conventional activated sludge process: Implications to water reuse

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HIGHLIGHTS

- MBR process is more effective than CASP in the removal of bacteriophages.
- SOMCPH proves to be more resistant to MBR treatment than FRNAPH.
- BFRAGPH are not always detectable in the pre-treated effluent.
- SOMCPH are the most suitable indicators to evaluate the MBR process performance.
- SOMCPH are the most suitable indicators to evaluate the safety of the MBR effluent.

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ABSTRACT

The effectiveness of a full scale membrane bioreactor (MBR) in the removal of bacteriophages and bacterial fecal indicators from municipal wastewater was compared with that obtained by conventional activated sludge process (CASP). Somatic coliphages (SOMCPH) and F-RNA specific bacteriophages (FRNAPH) were always detected in the pre-treated effluent (mean: $6Log_{10}$), while phages infecting *Bacteroides fragilis* were not always present (mean: $3.9Log_{10}$). The MBR process was able to achieve respectively 2.7 and $1.7Log_{10}$ higher reductions of SOMCPH and FRNAPH compared to CASP (significant differences: $P < 0.05$). SOMCPH were found to be the most suitable indicators for assessing MBR performance, since they showed greater resistance to biofiltration than FRNAPH and a more regular distribution in pre-treated effluent than BFRAGPH. Moreover, since the traditional bacterial indicators were almost totally removed by biofiltration, SOMCPH proved to be the best indicators to evaluate the microbiological risk when MBR effluent is discharged into natural waters or reused.

1. Introduction

Municipal wastewater is usually treated by a complex process that includes primary settling, biological degradation and secondary clarification. The efficiency of conventional activated sludge process (CASP) in removing pathogenic microorganisms has been investigated in several studies, which have concluded that these treatments may not be sufficient to achieve microbiologically safe effluent to be discharged into natural waters or to be reused (Koivunen and Heinonen-Tanski, 2005; Simmons and Xagoraraki, 2011; Zhang and Farahbakhsh, 2007). Notable pathogens common in secondary wastewater effluents include the environmentally resistant oocysts of *Cryptosporidium parvum,* cysts of *Giardia lamblia* and a variety of enteric bacteria and viruses. In order to reduce the potential microbiological risk, the secondary effluent is generally subjected to a further tertiary treatment by sand filtration (Zanetti et al., 2006), ultraviolet and ionizing radiation (Taghipour, 2004), or, more frequently, by chemical disinfection with chlorine, ozone, and peracetic acid (Chen and Wang, 2012; De Luca et al., 2008; Koivunen and Heinonen-Tanski, 2005; Zanetti et al., 2007). The generation of harmful disinfection by-products (e.g. THM) and the persistence of disinfection residues are considered adverse environmental effects of chemical disinfection processes (Chen and Wang, 2012; Wert et al., 2007), so that increased attention has been focused on the development of techniques alternative to the conventional activated sludge treatment.

The membrane bioreactor (MBR) is considered an effective, non-hazardous advanced treatment alternative (van Nieuwenhuijzen et al., 2008). MBR is a modification of CASP, in which separation of solids is achieved without the requirement of a secondary sedimentation in settling basins. Instead this function is carried

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out by a membrane, which retains the particulate phase within the reactor and allows the treated, clarified effluent to pass to the next process or to be discharged/reused. The small pore size of the membranes (0.4 μm) employed for separation of solids enables them to remove a wide range of microorganisms.

The efficiency of microbial removal by wastewater treatments is usually tested using traditional fecal indicators (fecal coliforms, *Escherichia coli*, enterococci) (USEPA, 2004; WHO, 2006). In recent years, information on the effectiveness of virus removal by sewage treatment processes has become of major concern, due to the epidemiological significance of viruses as waterborne pathogens and the large number of enteric viruses excreted in human feces (Simmons and Xagoraraki, 2011). Since current methods for the isolation and enumeration of viruses are quite expensive and complex, bacteriophages have been proposed as models of enteric viruses in microbiological water quality control (Grabow, 2001; IAWPCR, 1991; Leclerc et al., 2000). Bacteriophages are considered particularly suitable as viral indicators since they are commonly excreted in the feces of human and warm-blooded animals are as resistant as enteric viruses in aquatic environments, and have a similar replication mode, structure, composition, and size (Skraber et al., 2004). Furthermore, bacteriophages show higher resistance to treatment than bacterial indicators, thus representing a useful tool for evaluating the effectiveness of microbial removal by wastewater treatments (Arraj et al., 2005; Zanetti et al., 2007; De Luca et al., 2008). Methods for their detection and enumeration are simple, rapid, inexpensive and require no confirmation. The groups of phages proposed as indicators include Somatic coliphages (SOMCPH), F-RNA specific bacteriophages (FRNAPH) and phages infecting *Bacteroides fragilis* (BFRAGPH) (Contreras-Coll et al., 2002; Gantzer et al., 2001; Ebdon et al., 2007). *Bacteroides* are obligate anaerobic Gram-negative bacteria that constitute a major proportion of the bacterial flora of the human intestinal tract and bacteriophages infecting particular strains of *B. fragilis* appear to be found almost exclusively in fecal material of human origin (Ebdon et al., 2007). There is no indication that FRNAPH and BFRAGPH can multiply in the environment (Contreras-Coll et al., 2002). The F-specific coliphages can only reproduce in *E. coli* cells equipped with sex pili whose synthesis occurs at temperatures above 30 °C in the intestine of the homoiothome before the host bacteria are discharged into the water medium. Some SOMCPH may multiply in water environment, but, according to Muniesa and Jofre (2004), the conditions that support their multiplication are rarely formed in water environments and only 3% of the environmental non-fecal host-bacteria can support the replication of SOMCPH.

Further confirmation of the importance of bacteriophages as indicators of fecal contamination comes from the fact that SOMCPH have been included as fecal indicators in the regulations on the quality of drinking water in Québec (Lois et Régulements Québec (L.R.Q.), 2001) and in water quality guidelines such as the Ground Water Rule of USEPA (2006). Also in Italy, the requirements for the quality of drinking water (D. Lgs., 31/2001) include anti *E. coli* phages among the "supplementary" parameters, which must be applied only if deemed necessary by the Health Authority in charge of the control and vigilance of the quality of drinking water.

In recent years, various studies, performed first on pilot plants (Arraj et al., 2005; Ottoson et al., 2006) then also on full-scale municipal wastewater plants (Francy et al., 2012; Marti et al., 2011), have compared the efficiency of the MBR system with that of the conventional active sludge method in the treatment of municipal wastewaters.

In general, the efficacy of the two methods was compared on the basis of traditional bacterial indicators. However, considering the possible reuse of the wastewater for agricultural, industrial and civil purposes, such investigations should also examine the extent of their viral contamination. The aim of this work was to evaluate the effectiveness of a full-scale MBR system on microbial removal from municipal wastewaters, compared with that obtained by CASP. The removal of bacteriophages, as indicators of enteric viruses, was compared with that of traditional bacterial indicators, in order to obtain information on the efficiency of the membrane bioreactor to produce an effluent suitable for discharge into natural waters or for reuse. In addition, the effect of the wastewater treatments on different groups of phages was also studied in order to identify the most suitable indicator for assessing the performance of the MBR process.

2. Methods

2.1. Facility

The study was performed in a full-scale wastewater treatment plant with an overall potential of around 36,000 equivalent inhabitants. The plant mainly received domestic wastewater. Parallel to the line using a conventional activated sludge process (CASP), a second line was installed (potential of around 8,000 equivalent inhabitants) that uses MBR technology. The cycle for the purification of the incoming wastewater involved pre-treatment (large and fine screenings: 4 cm and 2 mm respectively and sand removal) after which the effluent was divided between the two lines.

The MBR consists of a tank fitted with 12 filtration modules made up of 400 cartridges which uses a flat-sheet submerged membrane (Kubota Corporation, Osaka, Japan), made of chlorinated polyethylene with a non-woven fibrous support (polyethylene terephthalate: PET) and giving a nominal pore size of 0.4 μm. Each membrane cartridge consists of a solid support plate (acrylonitrile butadiene styrene: ABS) with a spacer layer between it and a welded flat sheet membrane on both sides. The membrane cartridge is 1000 (H) × 490 (W) × 6 mm thick and provides an effective filtration area of 0.8 m^2. The total filtering surface is 3840 m^2.

Treated water permeates through the membrane sheet and spacers to come out via the nozzle into the top of the support plate and it is withdrawn and pumped along the submerged filtration modules. The modules are equipped with an air flow system which creates increased turbulence near the membranes, thus controlling the biomass accumulation at the membrane surface. Normal operation parameters are: flux = 21 L m^{-2} h^{-1}; trans-membrane pressure = 0.04 bar.

2.2. Sampling

Over a period of approximately six months, 18 sample collections were made, each consisting of three instantaneous samples from: 1. pre-treated effluent; 2. activated sludge effluent; 3. MBR permeate. A total of 54 samples were collected. The samples were kept at a temperature of 4 °C and were analyzed within 2–3 h. In all samples, in addition to *E. coli* (EC) and enterococci (ENT), somatic coliphages (SOMCPH), F-RNA specific bacteriophages (FRNAPH) and bacteriophages infecting *B. fragilis* (BFRAGPH) were enumerated as indicators of enteric viruses.

The organic load of the pre-treated effluent was evaluated measuring the Chemical Oxygen Demand (COD) (Zanetti et al., 2010).

2.3. Quantification of bacteriophages

Bacteriophages were enumerated with the double layer plaque assay, adopting the ISO methods, as previously described (Zanetti et al., 2010). The sample was mixed with a small volume of semi-solid nutrient medium (Oxoid, Milan, Italy), with an appropriate aliquot of an 18–20 h culture of host strain (*E. coli* ATCC