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Faecal bacteria and bacteriophage inactivation in a full-scale UV disinfection system used for wastewater reclamation

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Abstract A study was carried out to compare the inactivation of faecal bacteria and one type of bacteriophage in a full-scale UV disinfection system. The system is part of a water reclamation facility for effluent reuse in golf course and agricultural irrigation. Influent and effluent samples were taken over two sampling periods (three consecutive days in July and one day in August), with three different UV doses applied each day (ranging from 10 to 40 mW.s/cm^2 and 20 to 80 mW.s/cm^2 in July and August, respectively). Effluent samples were also taken from a chlorine disinfection channel (5 mg Cl_2/L dose) operating in parallel to the UV system. Total coliforms (TC), faecal coliforms (FC), faecal streptoccoci (FS) and somatic coliphages (SC) were measured in each sample. F-specific RNA bacteriophages and bacteriophages of *Bacteroides fragilis* were also measured one day in July. The decay ratio observed for all the microorganisms was similar when UV doses applied were low (July), ranging from 1.15 to 1.25 log-units. This suggests that bacterial indicators may be suitable for virus inactivation control when low UV doses are applied; however, such low doses are inadequate to achieve effluent quality requirements for unrestricted irrigation. At higher UV doses (August), decay ratios for TC and FC were 3.1 and 2.8 log-units respectively, indicating that they were more susceptible to UV exposure than SC and FS, with decay ratios of 2.6 and 1.0 log-units, respectively. Nevertheless, these higher doses were also inadequate to achieve water quality requirements for unrestricted irrigation. The decay ratio of SC during chlorine disinfection was clearly lower than that of the other microorganisms. Bacteriophages of *Bacteroides fragilis* were more resistant to UV disinfection than SC and F-specific RNA. In fact, bacteriophages of *Bacteroides fragilis* were not affected during UV exposure. A UV dose ranging from 40 to 80 mW.s/cm^2 marks the borderline beyond which inactivation rates of SC are clearly lower than those of bacterial indicators.

Keywords UV disinfection; faecal bacteria; bacteriophages; somatic coliphages; water reuse; water reclamation

Introduction

Water-borne infectious diseases are usually related to contaminated or untreated waters. However, many studies have shown that waters treated by supposedly effective conventional methods can also have a significant role in the transmission of disease-causing microorganisms (Bosch *et al*., 1991; Armon and Kott, 1995; Lucena and Jofre, 1996). These studies show that enteroviruses and protozoan parasites are the infectious agent commonly responsible for these outbreaks. This is not surprising, because enteroviruses and protozoan parasites differ from widely accepted microbial indicators, such as faecal bacteria, in terms of structure, morphology, biochemical composition, resistance and prevalence in water. Therefore, the usefulness of commonly accepted microbial indicators for the evaluation of disinfection processes is limited, because the absence of these bacteria can not ensure the absence of enteroviruses and protozoan parasites, which in any case are difficult to detect. Thus, if the priorities are an accurate evaluation of the effectiveness of disinfection processes and the concern for public health, it will be necessary to have well developed and standardised analytical methods for microbial indicators, that provide a

reasonably accurate measurement of the concentrations of enteroviruses and protozoan parasites present. In this context, some bacteriophages share several morphological and biochemical properties with enteroviruses, and new well developed methods for their detection and enumeration are available, offering the possibility of their use as indicators (Lucena and Jofre, 1996; ISO, 1996).

Three groups of bacteriophages are currently being evaluated as viral indicators: somatic bacteriophages, F-specific RNA bacteriophages, and bacteriophages of *Bacteroides fragilis* (IAWPRC, 1991). Somatic bacteriophages infect *Escherichia coli* and other related bacteria through the cell wall, and they have DNA. They are found in human and animal faeces, and are both the most numerous in wastewaters and the easiest to detect. F-specific RNA bacteriophages also infect *Escherichia coli* and other related bacteria, but throughout sex pili. They are also found in human and animal faeces. Bacteriophages of *Bacteroides fragilis* have DNA, and are only found in human faeces. They are the least numerous in wastewaters, and are also the most difficult to detect and enumerate (Lucena and Jofre, 1996).

Many studies have shown that viruses are more resistant to disinfection than faecal bacteria, and that these microorganisms have very different inactivation kinetics (Tree *et al.*, 1997; Yates and Gerba, 1998). Thus, there is a clear public health interest in considering viral inactivation, when evaluating wastewater reclamation projects, as opposed to simply complying with faecal bacterial requirements (Tree *et al.*, 1997). The relative efficiency of UV disinfection for inactivation of viruses and other microorganisms of concern in wastewater reclamation has been recently reviewed (Loge *et al.*, 1998). The results indicate that some bacteriophages are more resistant than enteric viruses, and that bacteriophages removal will in turn ensure removal of enteric viruses. Disinfection efficiency of UV systems depends on UV dose, influent microbial concentration, and water transmittance, the later being related to TSS and turbidity (Mujeriego *et al.*, 1998).

Sand filtration and chlorination of secondary effluents is a conventional water reclamation process for landscape and agriculture irrigation in Spanish Mediterranean areas (Eastern part of the country). However, UV disinfection is becoming increasingly popular, and many new reclamation projects include UV disinfection as preferred to chlorination. The effectiveness of these new reclamation projects is usually assessed and controlled using faecal bacteria, although there is a lack of information concerning bacteriophage inactivation. The objective of this study is to compare the inactivation efficiency of faecal bacteria and one type of bacteriophage (somatic coliphages) in a full-scale UV disinfection process, under exposure to different UV doses.

Materials and methods

The study was carried out in Castell-Platja d'Aro wastewater treatment plant (WWTP), which receives wastewaters from three tourist resort municipalities in the province of Girona (Catalonia, North-eastern Spain): Castell-Platja d'Aro, Santa Cristina d'Aro and Sant Feliu de Guixols. This coastal area has a Mediterranean climate, with dry summers (maximum monthly temperature of 25ºC) and mild winters (minimum monthly temperature of 8ºC). Annual rainfall ranges from 550 to 600 mm/year. The WWTP is operated by the Consorci de la Costa Brava, on behalf of the Catalan Wastewater Agency, and includes a conventional activated sludge biological plant followed by rapid sand filtration and disinfection by either UV or liquid chlorine. Maximum treatment capacity is 175,000 equivalent-inhabitants. The average winter flow is 8,700 m^3/d, while the average summer flow is 18,000 m^3/d. Annual average influent BOD_5 and TSS are 380 mg O_2/L and 610 mg/L, respectively. Annual average clarifier effluent concentration for BOD_5 and TSS were 13 mg O_2/L and 13 mg/L, respectively, in 1995 (Consorci de la Costa Brava, 1996).

The low-pressure UV disinfection system includes 3 banks of Phillips T UV 114 lamps, and is located in a channel 6.0 m long and 0.7 m wide. The first bank has 5 racks, of 6 lamps each, and the second and third banks have 7 racks, of 6 lamps each. The installed power of the first bank is 0.96 kW, producing a potential UV dose (assuming a 100% water transmittance at average flow) when performing alone of 30 mW.s/cm^2. The second and the third banks have an installed power of 1.34 kW each. When the first and second banks operate together, the potential UV dose is 80 mW.s/cm^2. The potential dose of the three banks operating simultaneously is 120 mW.s/cm^2. Disinfection performance was evaluated over a sampling period of 3 consecutive days in July 1999. The real UV dose applied each day was estimated through a table supplied by the manufacturer, where dose is a function of water flow rate and water transmittance. Water transmittance at 253.7 nm was measured using a Phillips PV 8620 UV/VIS/NR spectrophotometer, calibrated with a potassium dichromate solution (Mujeriego *et al*., 1998). As some of the lamps were not operational during the 3-day sampling period, the applied UV dose was estimated at 10 mW.s/cm^2 for first bank operating alone, 25 mW.s/cm^2 for first and second banks operating together, and 40 mW.s/cm^2 for all three banks operating together. An additional sampling session was conducted during one day in August 1999, to evaluate UV disinfection efficiency when all lamps were operational. The applied UV dose was 20 mW.s/cm^2 for the first bank, 50 mW.s/cm^2 for first and second banks, and 80 mW.s/cm^2 for the three banks. Grab samples were taken each day from the influent and the effluent of the system operating at a given UV dose. During the August sampling session, a sample was also taken from the effluent of the chlorine disinfection channel operating in parallel, and treating the same influent and at similar flow rates than the UV system. The chlorine dose was 5 mg Cl_2/L.

Total coliforms (TC), faecal coliforms (FC) and faecal streptoccoci (FS) were measured using the membrane filter technique as described by the Generalitat de Catalunya (1988). Somatic coliphages (SC) were grown by inoculating 1 mL of sample (or dilution of the sample) into tubes containing culture media (modified Scholtens' broth) and 1 mL of inoculum culture (*Escherichia coli* strain) used as cell host. The content of the tubes was poured into Petri dishes containing solid media, and incubated for 18 hours at 36°C, before SC were enumerated. A quality control of the host cells was run in parallel, to ensure the infective capacity of coliphages (ISO, 1998a).

Additional analyses of F-specific RNA bacteriophages and bacteriophages of *Bacteroides fragilis* were conducted by the Microbiology Laboratory of the Biology Department (Barcelona University) (Jofre and Lucena, personal communication) on samples taken during one day in August, to evaluate the relative presence of these phages as compared to that of SC. These analyses were carried out according to the methodology described in ISO (1996, 1998b).

Physical and chemical analyses were performed using the methods described in APHA-AWWA-WEF (1995).

Results

Table 1 shows the physical and chemical characteristics of the influent water to the UV disinfection unit during the July sampling period. Physical and chemical characteristics were very similar over the three days. Consequently, the results from the disinfection experiments have been evaluated using the average microbial concentrations over the 3-day sampling period. The effective operation of the sand filtration process resulted in low values for water turbidity and TSS.

Figure 1 shows the change of the average concentration of the four microorganisms evaluated, as a function of UV dose. Figure 2 shows the average microbial decay ratio (log C_1/C_2, where C_1 is influent concentration, and C_2 is effluent concentration) as a function of UV dose.

Table 1 Physical and chemical characteristics of influent water to the UV disinfection unit in the July sampling period

Parameter	1 July	2 July	3 July
Flow, m^3/h	240	250	240
Temperature, C	24.8	23.7	24.0
PH	7.6	7.6	7.8
Turbidity, NTU	0.5	0.5	0.5
Transmittance, %	57.1	58.9	59.4
TSS, mg/L	5	2	2

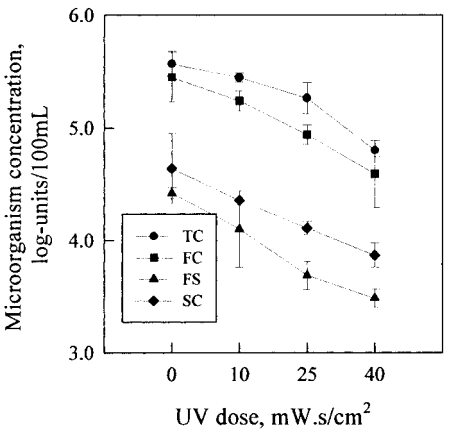

Figure 1 Changes of the average concentration of the four microorganisms evaluated, as a function of the UV dose during the July sampling period

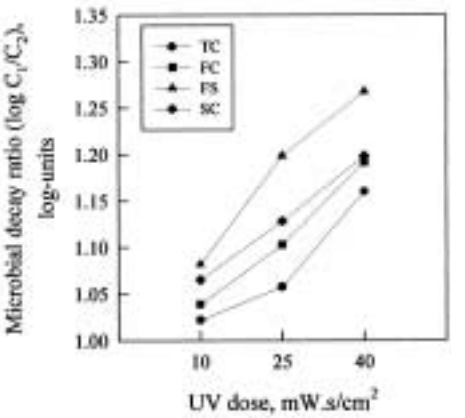

Figure 2 Average microbial decay ratio of the four microorganisms evaluated, as a function of UV dose during the July sampling period

TC and FC were more numerous (about one log-unit/100 mL) than SC, both in the influent and the effluent of the disinfection unit. Only FS had a lower concentration than SC (less than one log-unit). Inactivation of the four microorganisms showed a similar trend, as the UV dose increased, as indicated by the nearly parallel lines shown in Figure 1. Consequently, none of the four microorganisms appears significantly more sensitive to UV exposure than the others. The microbial decay ratio, when the system was operating at a UV dose of 40 mW.s/cm^2, was very similar for all the microorganisms, and ranged from 1.15 to 1.25 log-units. From the microbiological water quality point of view, the effluent of the UV disinfection system was not able to satisfy the water quality requirements for unrestricted irrigation.

The microbial decay ratio was lower for those microorganisms with higher influent concentration (Figure 2). This observation suggests that the decay ratio for a specific UV dose is a function of the microorganism influent concentration. Figure 3 shows the microbial effluent concentration as a function of the microbial influent concentration for each of the four microorganisms studied. The figure also shows the three regression lines corresponding to the three UV doses applied. Linear regressions were calculated considering all the microbial results corresponding to a given UV dose.

The correlation coefficient of the linear regressions was quite good for the three UV doses applied: $R^2 = 0.880$ for 10 mW.s/cm^2, $R^2 = 0.923$ for 25 mW.s/cm^2, and $R^2 = 0.838$ for 40 mW.s/cm^2, and indicate that there is a good linear relationship between influent and effluent microbial concentrations for the four microorganisms studied. Linear regressions are nearly parallel and indicate that UV dose is the factor controlling microbial effluent concentration, for a given influent concentration and a water transmittance value.

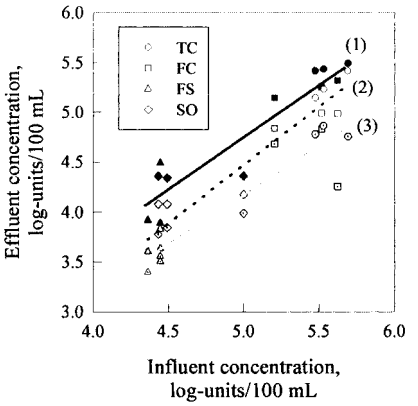

Figure 3 Effluent microbial concentration as a function of influent microbial concentration, during the July sampling period. Linear regression lines correspond to three UV doses: 1) 10 mW.s/cm^2, 2) 25 mW.s/cm^2, 3) 40 mW.s/cm^2

Table 2 shows the influent and effluent concentration of SC, F-specific bacteriophages and bacteriophages of *Bacteroides fragilis* in the samples taken on 3 July. Somatic and F-specific RNA bacteriophages had similar concentrations in the influent and the effluent. However, the decay ratio of SC was slightly higher than that observed for F-specific RNA bacteriophages. Bacteriophages of *Bacteroides fragilis* had clearly lower influent concentrations than the others, and do not show a detectable decay. These results suggest that bacteriophages of *Bacteroides fragilis* are less sensitive to UV inactivation than the other two bacteriophages.

Figure 4 shows the microbial decay ratio of the four microorganisms as a function of UV dose applied during the August sampling period. Water transmittance at 253.7 nm was 60%. Influent microbial concentration was higher than that of July (6.2 log-units/100 mL for TC, 6.3 log-unit/100 mL for FC, 5.1 log-units/100 mL for FS, and 5.2 log-units/100 mL for SC). As the UV dose applied reached 80 mW.s/cm^2, effluent microbial concentrations for TC, FC and SC (3.1 log-units/100 mL for TC, 3.5 log-units/100 mL for FC, and 3.6 log-units/100 mL for SC), were lower than in July, while FS concentration (4.1 log-units/100 mL) was higher than in July. The overall decay ratio of all the microorganisms in August was higher than in July, as a result of higher influent microbial concentrations, and usually lower effluent concentrations. Considering that environmental conditions were similar in July and August, the decay ratio differences must be attributed to the higher UV doses applied during August, when all the UV lamps of the system were operational.

The microbial decay ratio for the four microorganisms showed different trends as the UV dose increased. TC and FC were most sensitive to UV inactivation than FS and SC. These results indicate that differences in UV microbial sensitivity appear when UV dose increases. The decay ratios observed when the system was fully operational (UV dose of 80 mW.s/cm^2) were higher for TC and lower for FS. Decay ratios of FC and SC had inter-

Table 2 Influent and effluent concentrations, and decay ratios of bacteriophages measured in samples taken on 3 July. All expressed in log-units/100 mL

Bacteriophage	Influent	Effluent	Decay ratio
Somatic	4.43	3.79	0.64
F-specific RNA	4.13	3.81	0.32
Bacteroides fragilis	1.00	1.00	0.00

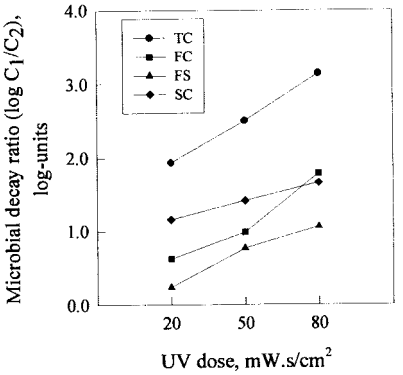

Figure 4 Average microbial decay ratio of the four microorganisms, as a function of UV dose during the August sampling period

mediate values, although FC seemed to be more sensitive than SC. From the microbiological water quality point of view, a UV disinfection system operating at a dose of 80 mW.s/cm^2 was not able to produce water that complies with the quality requirement for unrestricted irrigation.

In contrast to what was observed in July, the microbial decay ratio observed in August was not lower for the microorganisms with a higher influent concentration. These results suggest that influent microbial concentration has not much of an influence on decay ratio when UV dose exceeds a value around 80 mW.s/cm^2.

Microbial concentration in the effluent of the chlorine disinfection channel were: 0.0 log-units/100 mL for TC and FS, 0.8 log-units/100 mL for FC, and 2.9/100 mL for SC. Considering that influent concentrations were the same as those of the UV system, the decay ratio for SC was clearly lower than that observed when using UV disinfection, and indicates these phages are less sensitivity to chlorine disinfection than to UV disinfection.

Discussion and conclusions

Water shortages in the Mediterranean regions had made water reclamation and reuse an integral component of environmental programs. To ensure that effluents to be reused do not pose any unreasonable risk to public health and do comply with appropriate microbial quality requirements, disinfection processes are increasingly used, particularly UV disinfection systems. Disinfection process performance is usually measured by bacterial decay ratios. However, health risks associated with exposure to reclaimed water are frequently of viral origin. The inactivation of bacterial indicators and one bacteriophage (somatic coliphages, SC) has been evaluated in this study using UV disinfection and chlorine disinfection. At low UV dose (40 mW.s/cm^2), bacterial indicators and SC showed a similar decay ratio, ranging from 1.15 to 1.25 log-units. The decay ratio was clearly related to influent microbial concentrations, regardless of the specific microorganism considered. This suggests that bacterial indicators may be suitable for virus inactivation at low UV doses. However, these UV doses were not able to produce an effluent that satisfies the quality requirements for unrestricted irrigation. At a higher UV dose (80 mW.s/cm^2), TC were significantly more susceptible (3.1 log-units decay ratio) to UV inactivation than the other three microorganisms. FC, with a 2.8 log-units decay ratio, were more sensitive to UV inactivation than FS, with a 1.0 log-units decay ratio, and than SC, with a 2.6 log-units decay ratio. These results agree with previous studies indicating that SC are generally more resistant to disinfection than bacterial indicators (Lucena and Jofre, 1996). The SC decay ratio observed during chlorine disinfection was clearly lower than that observed for other

microorganisms. Consequently, bacterial indicators alone may not be suitable for demonstrating inactivation during wastewater UV disinfection (Tree *et al*., 1997). A UV dose of 80 mW.s/cm^2 was not able to produce water that complies with the quality requirement for unrestricted irrigation.

Three groups of bacteriophages are currently being evaluated as viral indicators. SC, F-specific RNA bacteriophages, and bacteriophages of *Bacteroides fragilis* were evaluated in this inactivation study. The results obtained confirm previous studies demonstrating that bacteriophages of *Bacteroides fragilis* are the most resistant to disinfection. It may be argued that bacteriophages of *Bacteroides fragilis* are too conservative as viral indicators, because they are less sensitive to inactivation than enteroviruses (Lucena and Jofre, 1997). However, rotavirus and hepatitis A virus are more resistant than enteroviruses to water treatments (Tartera *et al*., 1989).

The main conclusion of this study is that inactivation of SC and bacterial indicators reach similar values at low UV doses (40 mW.s/cm^2), and that inactivation becomes considerable different as the UV dose increases over 80 mW.s/cm^2, when bacterial indicators are more sensitive than bacteriophages. In contrast to the results obtained from UV disinfection, SC are considerably less sensitive to chlorine disinfection that common bacterial indicators. Finally, full-scale UV disinfection systems must be designed and operated to be able to inactivate both bacteria and viruses, in order to produce reclaimed water that satisfies currently accepted quality requirements for unrestricted irrigation.

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