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Bacteriophages as viral indicators for radiation processing of water: a chemical approach

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Abstract

Inactivation of the bacteriophages PHI X 174 (somatic coliphage), MS2 (F-specific coliphage) and B40-8 (phage infecting *Bacteroides fragilis*) suspended in tap water was studied applying gamma and electron beam irradiation as well. PHI X 174 phage was found to be a suitable viral indicator for water disinfection by means of ionizing radiation. The nutrient broths introduced simultaneously with the bacteriophages into the water when it is spiked with the phages for the experiments did not significantly change the scavenging capacity of the water matrix. No dose rate effect was observed with MS2 and B40-8 phages but PHI X 174 phage showed a clear dose rate effect. It was found that in water MS2 phage is significantly more sensitive to ionizing radiation than *Escherichia coli*.

Keywords: Bacteriophages; Inactivation; Water; Electron beam irradiation; Gamma irradiation

1. Introduction

At present, like untreated water, water treatment processes for disinfection are checked by fecal indicator bacteria although it is known that this method is not apt for controlling the virological quality of the treated water. Using bacteriophages instead of fecal bacteria as indicators for water quality control would meet both requirements. Especially 3 groups of bacteriophages (F-specific coliphages, somatic coliphages and phages infecting *Bacteroides fragilis*) have been proposed as viral indicators (Anonymus, 1991; European Community, Science, Measurements and Testing Program, 1999). For water disinfection applying 253.7 nm UV radiation appropriate investigations have been already performed (Sommer et al., 2001). For ionizing radiation as "disinfectant" there are just some data concerning gamma irradiation available (Sommer et al., 2001).

Radiation processing of water has been mainly considered for water remediation by pollutant decomposition. However, its potential for water disinfection becomes more and more attractive, e.g., disinfection of reclaimed wastewater by irradiation for irrigation purposes. For technical reasons and because of public acceptance electron beam accelerators have to be preferred to gamma radiation sources.

For gamma irradiation some data regarding a possible use of selected bacteriophages as viral indicators are already available. For electron beam irradiations, however, no data exists at all. As already mentioned radiation processing of water for disinfection will be most likely applied with electron beam accelerators. Therefore, main target of this study was to check the bacteriophages MS2 (F-specific coliphage),

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PHI X 174 (somatic coliphage) and B40-8 (phage infecting *B. fragilis*) for suitability as viral indicators regarding radiation processing of water by means of electron beam irradiation.

Moreover, the results obtained in a recent study (Sommer et al., 2001) regarding MS2 inactivation in tap water by gamma irradiation reveal an extremely low dose requirement for a virus system—it was even less than needed for a corresponding *Escherichia coli* inactivation. Consequently, it was strongly felt to verify these result and to repeat the gamma irradiation experiments.

To spike water with bacteriophage suspensions means automatically that a certain amount of nutrient broth used for phage propagation is added to the water. This addition certainly changes to some extend the characteristic of the water matrix with regard to its scavenging capacity for free radical species formed during irradiation and most likely responsible for the inactivation of the viruses. Therefore, another aim of this study was to assess the influence of the nutrient broths introduced, on virus inactivation by ionizing radiation.

Radiation processing will be most likely implemented by radiation chemists and civil engineers, respectively, and not by microbiologists. Accordingly in the present paper the matter is intentionally handled and presented like a chemical problem written for chemists and engineers. However, professional microbiologists and water hygiene experts performed all the microbiological experiments and controlled carefully the microbiological issue.

2. Experimental

Except otherwise stated all microorganisms investigated in this study (the bacteriophages MS2, B40-8, PHI X 174 and *E. coli*) were suspended in Vienna City tap water using initial concentrations of about 1×10^6 microorganisms/ml. The Vienna City tap water contains some natural solutes known to be effective as radical scavengers. These solutes and their concentrations are: 159 mg/l bicarbonate; 8 mg/l dissolved oxygen; 4.6 mg/l nitrate and 0.8 mg/l DOC.

Due to the known particle weight of MS2 and PHI X 174 which amounts to about 3.6 and 6.0 Megadalton, respectively, it is possible to calculate an approximate virus concentration in terms of g viruses per litre. The calculated concentrations are accordingly: 5.98×10^{-9} g/l for the MS2 phages and 9.97×10^{-9} g/l for the PHI X 174 phages. No data about the particle weight of B40-8 is available yet. These calculated concentrations are, of course, just a rough approximation and could describe at best the order of magnitude of the real virus concentrations.

Propagation and enumeration of the three bacteriophages MS2, PHI X 174 and B40-8 used in this study as well as the set-up for the gamma irradiation experiments have been already described elsewhere (Sommer et al., 2001). The gamma radiation source used was an AECL "Gammacell 220" Cobalt-60 source with an average dose rate of about 0.96 Gy s^{-1}.

The *E. coli* strains were cultured on Columbia agar (CM 331, Oxoid) for 24 h at 37°C. The enumeration of the bacteria as colony forming units (CFU) before and after irradiation was performed by pour plating with plate count agar (CM 325, Oxoid) and incubation at 37°C for 24 h.

Electron beam irradiations were performed with an ICT-500 electron beam accelerator (500 kV, 25 mA, scan width 1.2 m) manufactured by High Voltage Engineering, USA. For irradiation the phage suspension was pumped from a 2 l storage vessel through the irradiation chamber with a flow rate of 40 and 60 l/min, respectively. The electron beam entered the irradiation chamber through a 25 μm titanium window. The dimensions of the window were 5 cm (in the direction of the length axis of the beam) × 20 cm (in the direction of the water flow). Since the maximum penetration of the 500 keV electrons in water is just 1.4 mm the water layer in the irradiation chamber was adjusted to about 1 mm.

The electron beam doses were obtained by varying the beam current of the electron beam accelerator. Dose rate calibrations have been performed with different chemical dosimeters (Gehringer and Eschweiler, 1998). Accordingly, applied beam dose rates of about 400 up to 2000 Gy/s were obtained. All experiments were performed at room temperature. The temperature increase caused by irradiation was less than 2°C at the highest dose and, therefore, of no importance with regard to the inactivation of the test organisms.

Atrazine used as indicator to describe the scavenging behaviour of the water matrix was determined by gas chromatography/mass spectroscopy after solid phase extraction from water using C-18 columns.

3. Results and discussion

We repeated the gamma irradiation experiments for the inactivation of the three bacteriophages in tap water under exactly the same conditions as described by Sommer et al. (2001) and got identical results with all three phages. Fig. 1 shows the inactivation curves of the three bacteriophages in tap water obtained with another series of gamma irradiation in comparison to the inactivation of *E. coli* under identical conditions. The results confirmed the low dose requirement for MS2 phage inactivation and, moreover, the uselessness of

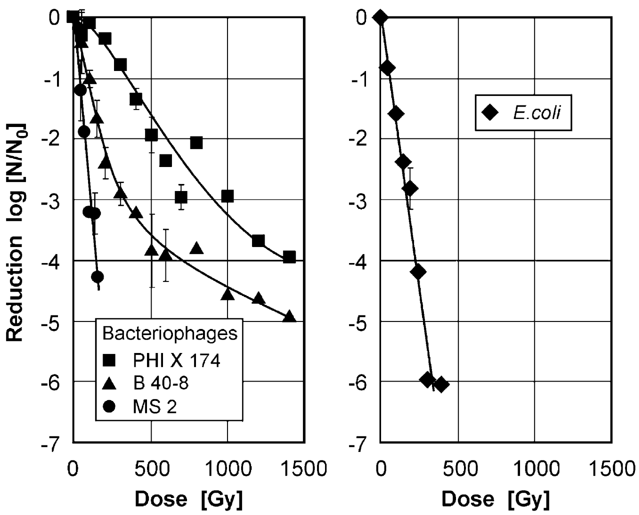

Fig. 1. Inactivation of the bacteriophages MS2, B40-8 and PHI X 174 as well as *E. coli* in tap water by gamma irradiation as a function of the absorbed dose.

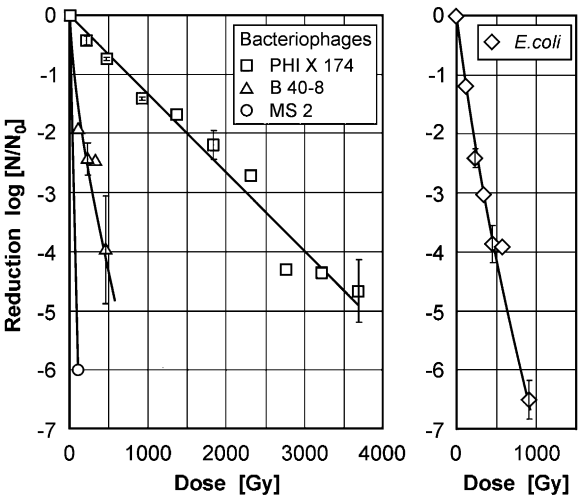

Fig. 2. Inactivation of the bacteriophages MS2, B40-8 and PHI X 174 as well as *E. coli* in tap water by electron beam irradiation as a function of the absorbed dose.

E. coli, respectively, to control the efficacy of water disinfection regarding its virological quality.

The results of the electron beam irradiation experiments (Fig. 2) confirmed the results obtained with gamma irradiation regarding the relative radiation sensitivity of the three phages (Fig. 1) and, of course, again the uselessness of *E. coli* and fecal coliforms, respectively, to control the virological quality of water after disinfection. However, there is an essential difference as compared with gamma irradiation. Relative to gamma irradiation, MS2 phage was found to be even more sensitive and PHI X 174 phage revealed to be considerably more resistant to electron beam irradiation while B40-8 resulted in almost the same response to both gamma and electron beam irradiation. Expressed in terms of dose rate under the conditions given a dose rate effect clearly occurred with PHI X 174 and certainly no dose rate effect was recorded with B40-8. The result obtained with MS2 phage showed at a first approximation a "reverse" dose rate effect, i.e., electron beam irradiation is more effective than gamma irradiation, the opposite is usually observed in aqueous systems. However, the inactivation curve for MS2 phage obtained with electron beam irradiation is just based on one single quantifiable result of measurement because of its high sensitivity to ionizing radiation. (All experiments performed at doses above about 115 Gy indicated values below the limit of detection.) The adjustment of the beam current of the electron beam accelerator used for such a low dose as 115 Gy is rather difficult and consequently quite inaccurate. Accordingly, it is not really possible to make a quantitative statement regarding the influence of the dose rate in connection with MS2. Most likely there is no real dose rate effect with MS2 inactivation by ionizing radiation.

The existence of a dose rate effect with PHI X 174 can be interpreted as a clear indication that inactivation is mainly caused by the free radical species generated by water radiolysis, i.e., by the so-called indirect effect of the ionizing radiation. This could be expected because of the rather low initial concentrations (relative to ng/l) of the viruses in water (Experimental), on the one hand. On the other hand, this is corroborated by results obtained with gamma irradiation when the bacteriophages were suspended in deionized water (Fig. 3). Under these conditions for all three phages lower doses were needed for the same extent of inactivation as compared to inactivation in tap water. In deionized water just the scavengers contained in the broths of the

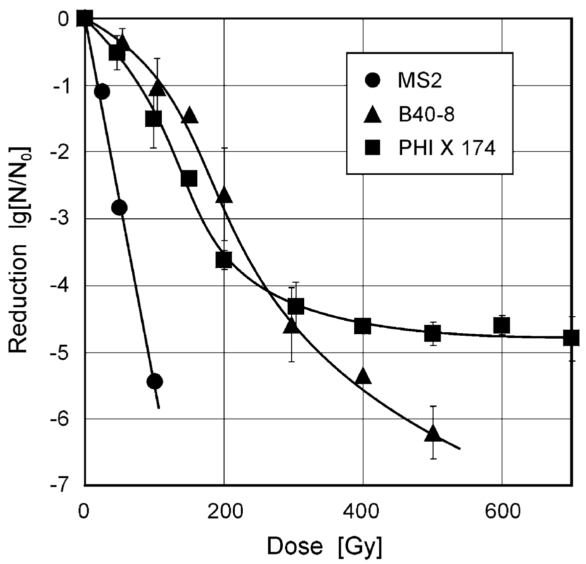

Fig. 3. Inactivation of the bacteriophages MS2, B40-8 and PHI X 174 in deionized water by gamma irradiation as a function of the absorbed dose.

different bacteriophages are present, together with some oxygen, while in tap water, beside oxygen, some other of the natural solutes present also act as radical scavengers. Consequently, due to the higher amount of radical scavengers in the tap water the dose requirement for the same amount of inactivation must be higher—a clear indication for an indirect effect.

These findings are in accordance with the literature where some authors attribute the inactivation of viruses in water by means of ionizing radiation to an indirect effect (e.g., Epe et al., 1988; Tölgyessy et al., 1988; Kuipers et al., 1998).

The experiments in deionized water, as the suspending medium, were not sufficient to clarify the influence of the individual nutrient broths on the results obtained. Therefore, additional experiments were performed using atrazine as an indicator instead of the microorganisms to figure out the scavenging capacity of the different broths used. Atrazine reacts quite efficiently with OH free radicals—the corresponding reaction rate constant is $3 \times 10^9\,dm^3/mol/s$—and should therefore be apt for elucidating the scavenging behaviour of the different broths after introduction into the tap water.

Before discussing the results obtained with atrazine as an indicator, some considerations regarding the scavenging capacity of the known substances contained in or introduced into the tap water should be taken into account. With regard to the scavenging of OH free radicals just the bicarbonate can be considered. According to its concentration of 159 mg/l its scavenging capacity was calculated to be $2.55 \times 10^4\,s^{-1}$. As regards the scavenging of the solvated electrons there is a contribution from the oxygen and the nitrate as well. Moreover, together with the broths there is also some chloroform in concentrations of about 3 mg/l added to the water. The resulting overall scavenger capacity for the solvated electrons was calculated to be $7 \times 10^6\,s^{-1}$. Although not knowing the contribution of the broths from these calculations it is likely that the contribution of the solvated electrons to the inactivation of the bacteriophages is small compared to the contribution of the OH free radicals. This is not only due to the expected higher scavenging of the solvated electrons by the water matrix but also due to their lower efficiency in the reactions with DNA, RNA and proteins that is reflected by lower reaction rate constants when compared to OH free radicals (von Sonntag, 1987).

Decomposition of 50 μg atrazine in tap water containing the different broths used with the bacteriophages by gamma and by electron beam irradiation is shown in Fig. 4. The data obtained follows approximately first-order kinetics. Accordingly, they are also applicable to the concentration range the bacteriophages were applied. It is, moreover, also possible to define a D_{10} value with atrazine meaning the dose necessary to reduce the atrazine concentration by a factor of 10. These atrazine results reflect the scavenging of the free radical species formed during water radiolysis by the water matrix used in the experiments with the bacteriophages. It is obvious that in all three cases a dose rate effect clearly exists. Accordingly, the bulk suspension obtained with the bacteriophages in tap water cannot be classified as a highly scavenged system. Consequently, the dose rate effect observed with PHI X 174 and the lack of a dose rate effect observed with B40-8 and most likely with

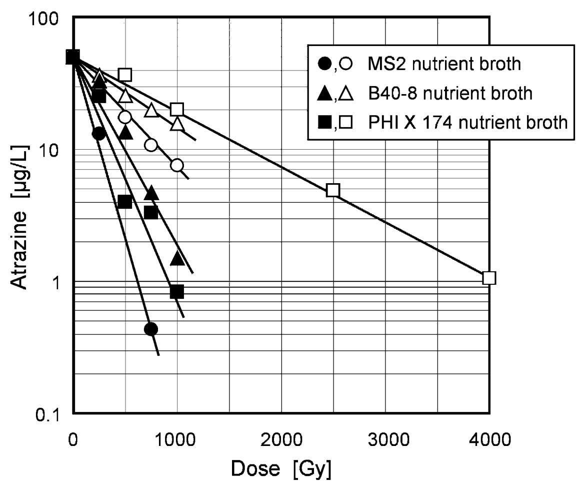

Fig. 4. Decomposition of atrazine in tap water containing the respective nutrient broths of the bacteriophages MS2, B40-8 and PHI X 174 by gamma (full symbols) and electron beam (open symbols) irradiation, respectively, as a function of the absorbed dose.

Table 1
D_{10} values for the inactivation of the bacteriophages MS2, B40-8 and PHI X 174 as well as for the decomposition of atrazine in Vienna City tap water

D_{10}-values in Gy				
Broth	Bacteriophages		Atrazine	
	Gammas	Electrons	Gammas	Electrons
PHI X 174	340	700	580	2500
B40-8	100	100	740	2020
MS2	45	20	380	1200

MS2, respectively, has to be attributed to the innate sensitivity of the microorganisms to radical attack and is not caused by different water matrices resulting from introduction of the various broths used with the bacteriophages.

The high sensitivity of the different bacteriophages to ionizing radiation compared to atrazine decomposition is clearly demonstrated when the D_{10} values obtained with atrazine are compared with the D_{10} values found with the bacteriophages (Table 1). Most remarkably in this respect is the high sensitivity of all three bacteriophages to electron beam irradiation. Comparing the electron beam D_{10} values of the bacteriophages inactivation with the corresponding D_{10} values of the atrazine decomposition the dose requirement for PHI X 174 inactivation is lower by a factor of about 3.5, for the B40-8 inactivation by a factor of about 20 and for the MS2 inactivation even by a factor of about 60. The nutrient broth introduced with the MS2 phage obviously caused less of an increase in the scavenging capacity of the water matrix than the other two (Fig. 4), but the difference is just a factor of about 2, i.e., this difference cannot be the reason for the high sensitivity of MS2 phage to electron beam irradiation. It was, moreover, observed that the MS2 inactivation curve obtained with electron beam irradiation in tap water (Fig. 2) is almost identical with the inactivation curve obtained with gamma irradiation in deionized water (Fig. 3). Consequently the effect of the water matrix regarding MS2 phage inactivation seems to be rather low most likely due to the high sensitivity of the MS2 bacteriophage to radical attack.

To illustrate the high sensitivity of the MS2 phage in water to electron beam irradiation a comparison with *E. coli* might be useful. The D_{10} value of *E. coli* inactivation in tap water obtained with electron beam irradiation was determined to be about 100 Gy (Fig. 2). The corresponding value for MS2 phage is about 20 Gy (Table 1). However, calculating the *E. coli* concentration applied in the experiments on the basis of 2×10^{-12} g/cell an *E. coli* concentration of about 2 mg/l results, i.e., several orders of magnitude higher than for the MS2 phage which was estimated to be in the range of sub-micrograms. Accordingly, irrespective of a concentration higher by almost 6 orders of magnitude (in terms of mass/litre) the D_{10} value of *E. coli* is even about 5 times higher as compared to MS2 phage—a clear indication for the exceptional high sensitivity of MS2 phage to radical attack. This is further accentuated by Lemke and Sinskey (1975) who stated that it is well established that vegetative bacteria are more radiation sensitive than viruses.

Moreover, with gamma irradiation as shown in Fig. 4 the scavenging capacity of the nutrient broth used with B40-8 phage was higher than that used with PHI X 174 phage. Nevertheless, the D_{10} value for the B40-8 inactivation with gamma irradiation is less by a factor of about 3.4 as compared to PHI X 174 phage. With electron beam irradiation the scavenging capacity of the nutrient broths used with these two phages was found not to be very different, just a factor of about 1.2 (Table 1). However, the corresponding D_{10} values for inactivation differ by a factor of 7. Consequently with phage B40-8 the effect of the scavengers present in the water with regard to phage inactivation was found to be relatively small.

PHI X 174 phage represent the only one in this study which showed an usually observed dose rate effect with respect to their inactivation in tap water. Even in this case the D_{10} values for the inactivation are less for both types of radiation when compared with the corresponding D_{10} values found with atrazine, indicating a higher radiation sensitivity of the bacteriophages than might be expected from the scavenging experiments. However, despite of the dose rate effect the difference in the D_{10} values of PHI X 174 phage and atrazine is much more pronounced with electron beam irradiation than with gamma irradiation (Table 1), another indication that electron beam irradiation is very efficient for phage inactivation in water. In any case, this phage is by far the most resistant of the three phages investigated in this study.

4. Conclusions

Among the 3 bacteriophages investigated PHI X 174 phage would be best apt for use as indicator virus to control the virological quality of water treated by means of ionizing radiation.

There is a clear indication that under the conditions given OH free radicals are responsible for virus inactivation. The nutrient broths introduced together with the bacteriophages into the water when performing the experiments increased the scavenging capacity of the respective water but did not result in a highly scavenged system. Thus, the lack of a dose rate effect observed with MS2 and B40-8 is due to the specific sensitivity of the bacteriophages to radical attack and not caused by the water matrix.

MS2 phage inactivation occurred already at extremely low radiation doses. The dose requirement was even significantly lower than for the corresponding *E. coli* inactivation. Accordingly for phages similar to MS2 phage the use of fecal indicator bacteria would also control inactivation of this type of viruses. Poliviruses, for instance, are similar with MS2 phage in size and structure. Experiments dealing with photocatalytic inactivation of coliform bacteria and viruses in secondary wastewater effluents (Watts et al., 1995) have shown that poliovirus 1 is inactivated more rapidly than coliform bacteria using titanium dioxide photocatalysis under sunlight and the simulated sunlight of F40BL lamps. In the present case photocatalytic inactivation is based on OH free radical attack to viruses and bacteria, respectively. Consequently, there is an indication that radiation processing of water for inactivation of coliform bacteria could simultaneously inactivate polioviruses sufficiently if contained in water.

However, taking into account the big difference between the radioresistence of phage PHI X 174 and *E. coli* our results confirm the fact that viruses may be more resistent to inactivation than fecal indicator bacteria. Thus, there is a need for developing alternative concepts, e.g., based on the use of bacteriophages, to survey the viral quality of treated water. Applying ionizing radiation for water disinfection in general, PHI X 174 phage has proven to be the best appropriate viral indicator under the conditions given.

Acknowledgements

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