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Secondary Effluent Disinfection: PAA Long Term Efficiency

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The paper summarizes the results of a bench-scale study to evaluate the long-term disinfection efficiency of peracetic acid (PAA). Bacterial counts were repeated 5, 24, and 29 h after the end of the disinfection test, to simulate real re-growth conditions (no residual quenching) and, for the 5 h interval, the potential re-growth (quenching of residual PAA). Fecal coliforms, *Escherichia coli*, and total heterotrophic bacteria (THB) were enumerated by traditional plate count technique; THB were also enumerated by cytometry. After disinfection, the residual PAA concentration became negligible in about 5 to 11 h, depending on the tested doses. Microbial counts showed that no appreciable re-growth took place after 29 h for coliform group bacteria. For THB, the previously cited enumeration techniques gave different results in re-growth tests, especially for the lowest PAA doses. Indeed plate count technique evaluates the ability to form colonies, while cytometry enumerates intact membrane cells. No re-growth took place, even when no residual disinfectant was present, suggesting that bacteria are unable, even at the lowest doses, to repair damage caused by the PAA disinfecting action. PAA was found to be an efficient disinfecting agent, not only as a bacteriostatic, but also as a bactericide.

Introduction

Wastewater disinfection ensures the elimination or inactivation of potentially disease-causing microorganisms such as bacteria and viruses; the main treatment objectives are public health and environmental protection. Microbiological standards for discharge into bathing areas and for wastewater reuse are enforced in several countries; for instance, for discharging into bathing areas, Italy requires a double standard: 2000 CFU/100 mL for total coliforms and 100 CFU/100 mL for fecal coliforms. For wastewater reuse, unlike other countries which adopt total and fecal coliforms as reference microorganisms, recent Italian rules establish a limit for unrestricted irrigation, which applies for all types of irrigation. This limit is set with reference to *Escherichia coli*, enforcing a 10 CFU/100 mL standard, with a maximum occasional value of 100 CFU/100 mL. Peracetic acid (PAA) disinfection can be a feasible alternative to chlorination due to the bactericidal, virucidal, fungicidal, and sporicidal effectiveness of PAA (*1*−*8*). Moreover PAA is not quoted for DBPs production (*9*−*11*). On the other hand the potential microbial re-growth due to acetic acid (which is present both in the PAA commercial solution and as a product of its decomposition) is one of the possible disadvantages associated with PAA disinfection.

Crop irrigation is a discontinuous practice, and irrigated fields may be quite far from the wastewater treatment plant (WWTP) discharge. Therefore, the reclaimed water at the point of use may display a different microbial content from the reclaimed water just after disinfection. The potential bacterial re-growth and the real bactericidal properties of the disinfecting agent (meaning its ability to kill bacteria) can be assessed by studying the long-term disinfection efficiency. PAA long-term efficiency is still not thoroughly understood. Lazarova et al. (*12*) observed microbial re-growth in wastewater disinfected with PAA, diluted with seawater, 2 days after disinfection; another study (*8*) concluded that no re-growth takes place in the first few hours after the disinfection.

Another key issue for a correct disinfection efficiency assessment through a determination of microorganisms, is the analytical method used. Assessment of disinfection efficiency is traditionally based on the elimination of fecal indicators such as total coliforms, fecal coliforms, and fecal streptococci. New methods such as cytometry (both static and flow), can provide information on cell viability and on the number of stressed bacteria, which are viable, but not cultivable (*13*−*14*). Among the main kinds of stress caused by chemical disinfection, is the loss of cultivability; non-cultivable microorganisms can keep their pathogenicity (*15*) and, therefore, be dangerous if spread in the environment.

The main purpose of this work was to study long-term efficiency of PAA, evaluating the evolution of microbial counts over time after disinfection, both in the real situation of active residual disinfectant decay and in the condition of maximum potential re-growth, when residual PAA is quenched. The effect of the disinfectant is described according to the results obtained by different methods (enumeration on plate count agar and by cytometry).

Materials and Methods

Bench-scale assays were carried out on the secondary effluent of a conventional municipal WWTP of the greater Milan area, preliminarily filtered at pilot scale (rapid sand filtration: grain size = 0.8−1.2 mm, D_{10} = 1 mm, filtration rate = 10.6 m/h). Four different PAA doses, ranging from 2 to 15 mg PAA/L, three contact times (HRT = 12, 18, 36 min), and three re-growth times (t_r = 5, 24, 29 h) were tested, adopting fecal coliforms (FC) and *E. coli* as reference microorganisms. Total heterotrophic bacteria (THB) were also enumerated.

The 5 h lag was chosen to simulate the time needed to transfer reclaimed water from the plant to the irrigation area. The 24 h time interval simulated a daily storage tank and the 29 h interval was the sum of the above-mentioned times.

Disinfection tests were performed in a completely mixed batch reactor (V = 5 L), by a mechanical mixer at room temperature (from 20 to 22°C). No additional temperature control was performed on filtered water samples, whose temperature increased gradually from the values shown in Table 1 to room temperature. For re-growth tests, after disinfection, samples were maintained in unmixed closed sterile glass bottles at constant room temperature (20−22°C) for the reference re-growth times (t_r). For the 5 h re-growth time, tests were performed in two different ways with respect to PAA residual active concentration, to evaluate both the "potential" (quenching residual PAA by sodium thiosulfate 0.1 N) and "real" (no residual quenching) conditions. Room temperature was higher than in-field (10.8−12.3°C in 95%

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TABLE 1. Main Physico-chemical Characteristics of the Filtered Water before Disinfection

parameter		samples no.	min	max	mean	standard error	95% confidence range
temperature	°C	42	9	22	11.5	0.3	10.8−12.3
pH		44	6.4	7.3			
turbidity	NTU	44	1.2	11	3.5	0.5	2.5−4.5
TOC	mg/L	43	4.0	14	7.8	0.4	7.0−8.5
TSS	mg/L	43	0.2	11.0	2.7	0.38	2.0−3.5
absorbance[a]	cm^{-1}	44	0.116	0.426	0.205	0.010	0.183−0.227
fecal coliforms	10^3 × CFU/100 mL	43	2	740	103	25	53−153
E. coli	10^3 × CFU/100 mL	43	1	393	50	13	23−77
THB (by PCA)	10^3 × CFU/100 mL	31	30	2,048	343	78.0	184−502
THB (by cytometry)	10^3bacteria/mL	11	4,000	24,600	11,800	2,490	6,250−17,350

[a] At 254 nm (optical path: 1 cm).

confidence interval, see Table 1), but corresponded to the optimal level for most environmental bacteria and was representative of the in-field conditions that could be expected during irrigation seasons.

Each combination of dose, contact time and re-growth time (D-HRT-t_r) was repeated 4−8 times. Active PAA doses were controlled by titrating the mixture concentration before use.

Filtered water before disinfection was analyzed for pH, turbidity, TSS, TOC, UV absorbance at 254 nm (optical path 1 cm), and the previously mentioned microbial indicators. The measured values are summarized in Table 1. Reclaimed water after disinfection and samples for re-growth evaluation were analyzed for pH, TOC, and microbial indicators; residual disinfectant concentration was also measured by applying the DPD colorimetric method, as reported below (*16*). To evaluate organic content, TOC and BOD_5 were measured at the beginning of the experimental work, but as BOD_5 values were always below the analytical detection limit (10 mg/L), only TOC was later analyzed. All samples were analyzed immediately after collection.

pH, turbidity, TSS, TOC, UV Absorbance. Analyses were performed according to American Standard Methods (*17*).

PAA. PAA technical-grade solution was supplied by Air Liquide Italia SpA (% w/w of peracetic acetic: 15, acetic acid: 17, hydrogen peroxide: 23). Its concentration was determined by titration. PAA residual concentration was estimated by applying the DPD colorimetric method based on absorbance measured at 530 nm after H_2O_2 decomposition by catalase and after addition of DPD, phosphate buffer solution (pH 5.5) and KI for catalyzing the reaction. This methodology has been used in previous studies (*16*).

Microbial Enumeration. The count of coliforms was made using membrane filtration technique (methods 7020B and 7030C, ref *18*). For fecal coliforms (FC), membranes were incubated for 24 h at 44°C on C-EC Agar (Biolife, Italy). *E. coli* colonies were found, on the same plate of FC, fluorescent under the light of a Wood lamp (365 nm).

Total heterotrophic bacteria (THB) were enumerated in two different ways: (a) by PCA, inoculating plate count agar culture media and counting colonies after 7 days' incubation time at 28°C and (b) by cytometry. DNA double staining by Sybr Green I, SG (Molecular Probes, Eugene, Oregon) and Propidium Iodide, PI (Sigma, St. Louis, Montana) (*13*) was used in order to select intact, damaged, and dead cells. Both pigments make specific bonds with microbial DNA, but they penetrate the cell through plasmatic membranes in different ways: SG can penetrate both intact and dead cells, while PI penetration concerns only compromised membrane, damaged or dead bacteria. Therefore, dead cells emit only red fluorescence, intact cells only green fluorescence and damaged cells both green and red, according to the amount of PI which can penetrate the cell, which in turn depends on the extent of damage. Cytometry analysis by-passes the problems arising from cultivability and, consequently, detects a higher number of bacteria than PCA counts. The differences observed between PCA and cytometry counts (about 2 orders of magnitude) in the filtered water fed to PAA disinfection is in agreement with literature data (*19*−*20*).

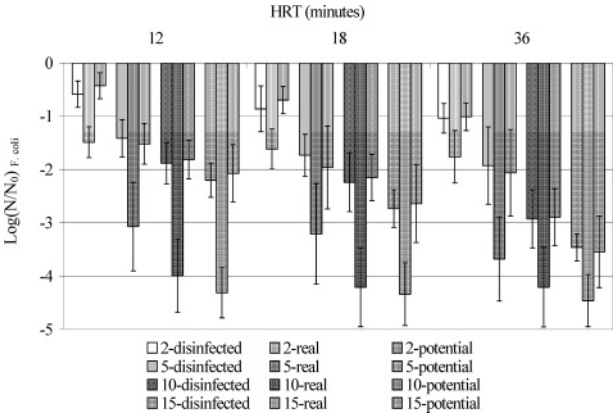

FIGURE 1. Mean log-reduction of fecal coliforms, with the 95% confidence interval, in PAA disinfected effluent (disinfected), quenched PAA disinfected effluent after 5 h (potential), and PAA disinfected effluent after 5 h (real) as a function of disinfection contact time and PAA dose (values, expressed as mg/L, are reported in the legend). *N* stands for N_d for disinfected samples and N_r for real and potential re-growth samples.

In the past bacterial counts by cytometry have not been performed on coliform group bacteria because a standardized, reliable method able to detect them specifically has not been available.

Results and Discussion

Fecal Indicators. Disinfection tests showed that PAA efficiency varies with the dose, but is relatively independent from contact time. Over 12 min, the increase of contact time does not involve any further bacterial abatement (*21*), as shown in Figure 1 for fecal coliform (bars named disinfected).

The role of residual PAA in preventing bacterial re-growth was studied by comparing microbial counts 5 h after the end of the disinfection treatment in samples with active residual PAA and in samples with quenched residual PAA. Those results were then compared with the ones obtained immediately after disinfection, as shown in Figure 1. All the data were normalized as log-reduction with respect to N_0 (number of bacteria enumerated in the influent samples). This approach permitted the quantification of the efficiency of the whole process 5 h after disinfection, i.e., the effects of disinfection itself and those coming from the subsequent disinfecting action, deriving from the residual active concentration of PAA and the possible re-growth phenomena in

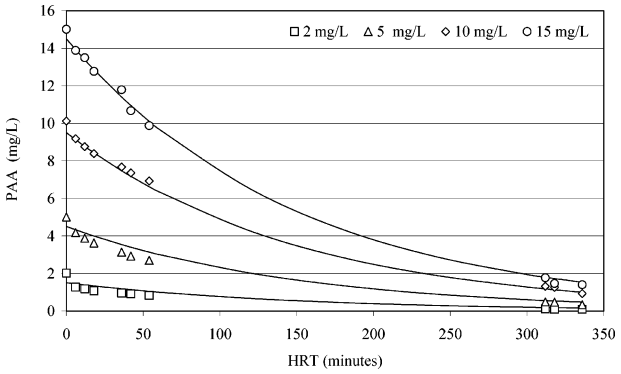

FIGURE 2. PAA residual concentration versus time: mean of experimental data (dots) and 1st order kinetics (line) derived from eq 2 and parameters in Table 2.

the reservoir and along the irrigation distribution system of reclaimed disinfected water. This can be described by the following:

$$\mathrm{Log}\left(\frac{N_r}{N_0}\right) = \mathrm{Log}\left(\frac{N_d}{N_0}\right) + \mathrm{Log}\left(\frac{N_r}{N_d}\right) \quad (1)$$

where N_d is the number of microorganisms surviving disinfection and N_r is the number of microorganisms after a fixed re-growth time. Regrowth log (Log N_r/N_0) can in fact be seen as the sum of two terms, taking into account the inactivation which occurred during disinfection (Log N_d/N_0) and the microbial inactivation after leaving the disinfection reactor (Log N_r/N_d).

When residual active PAA was not quenched, the disinfection process went on after the contact time. Depending on PAA initial dose and contact time (HRT), log-reduction increased by about 1−2.5 units (Log N_r/N_d), compared with the results obtained just after disinfection (Log N_d/N_0), as can be observed for fecal coliform in Figure 1. Without any active residual PAA (quenched samples), log-reduction obtained immediately after disinfection were comparable to that obtained 5 h later for all the doses and contact time tested (referred to as *disinfected* and *potential* in Figure 1). The same trend was found for *E. coli*: log-reduction increased by about 1−2 units, with the higher value limited by an almost complete disinfection after HRT. This suggests that no real re-growth took place, even when no residual disinfectant was present. These findings suggest that bacteria are unable to repair the damage caused by PAA action, at least during the first 5 h after the depletion of residual disinfectant, even at the lowest doses. These results confirm the bactericidal efficiency of PAA in addition to its bacteriostatic properties (i.e., its inhibiting effect on bacterial growth).

Usually disinfectant residual active concentration is not quenched in WWTP, so bacterial re-growth was also studied at longer re-growth intervals, as previously mentioned.

First, the depletion kinetics of the residual PAA concentration at the end of disinfection tests, due both to natural decay (*21*−*22*) and oxidative consumption, has to be identified to quantify its availability in the reservoir/distribution system. PAA decays according to a 1st order kinetics, as shown in Figure 2, modified with a term of initial oxidative consumption (D) as proposed by Haas and Finch (*23*):

$$\mathrm{C} = (\mathrm{C}_0 - \mathrm{D})e^{-k \cdot t} \quad (2)$$

PAA concentrations after 24 and 29 h were below the detection limit of the analytical method (0.17 mg/L), and consequently, are not shown in Figure 2 and were not used to fit the above-reported kinetics. The least-squares estimates of models' parameters are summarized in Table 2. The initial oxidative

TABLE 2. Disinfectant Concentration Decay Kinetics: Estimated Parameters

	D	k
estimate	0.494	6.7E−03
conf. 95%	0.360−0.628	6.2×10^{-03} to 7.3×10^{-03}
std. err. (S. E.)	0.0681	0.0003
t (df)	7.2527	23.0085
p-level	$<1 \times 10^{-30}$	$<1 \times 10^{-30}$
R^2	0.967	
Data no.	269	

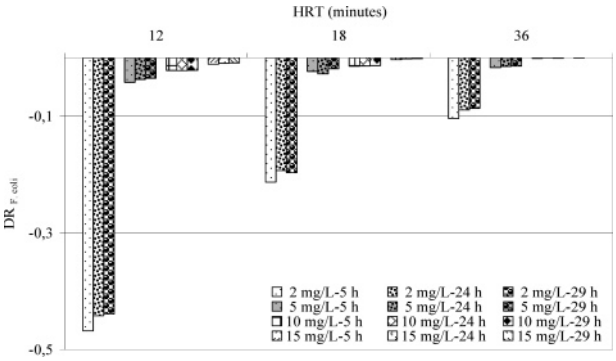

FIGURE 3. Degree of reactivation for fecal coliforms versus different initial PAA doses and re-growth time at selected disinfection contact time (the legend gives the pairs of data: PAA dose−re-growth time).

consumption, estimated as 0.494 mg PAA/L, was significant only for the lowest applied dose (2 mg/L), which is, however, the most used dose in Italy, when the effluent disinfection is intended for discharge into surface water and not for reuse (*22*). It would appear that PAA depletion in a well nitrified and filtered effluent, such as the one used in this experimental work, is therefore, mainly due to natural decay.

The almost total disappearance of PAA within 5−11 h after disinfection, depending on the initial doses, suggests that any additional inactivation effects occurring with longer re-growth times (24 and 29 h) are due to secondary effects of PAA (i.e., disinfecting effects occurring when residual PAA was absent). In fact, the effects of PAA damage can increase with time and cells seem to be unable to repair. This would explain the fact that, despite the increased availability of readily biodegradable carbon (acetic acid), due to PAA decomposition, in most cases microbial counts decrease with time, and in no case do they increase.

An alternative way to quantify re-growth is the index recommended by Kelner (*24*) for UV photoreactivation (DR, degree of reactivation), which can be expressed as the following:

$$\mathrm{DR} = \frac{N_\mathrm{r} - N_\mathrm{d}}{N_0 - N_\mathrm{d}} \quad (3)$$

where the symbols have the meaning previously defined. It represents the fraction of the initially inactivated cells ($N_0 - N_\mathrm{d}$) that are subsequently reactivated ($N_\mathrm{r} - N_\mathrm{d}$). A positive DR indicates that there is re-growth, while it is ~0 both if $N_\mathrm{r} = N_\mathrm{d}$ (no re-growth takes place after disinfection) or if $N_0 \gg N_\mathrm{d}$ (a high level of disinfection is reached so that, from a mathematical point of view, any possible further increase of N_r with respect to N_d is not appreciable). Mean values of DR for fecal coliforms are reported in Figure 3.

The experimental results showed that there was no significant re-growth for fecal coliforms. The great difference among DR values at 2 mg/L and those displayed at higher

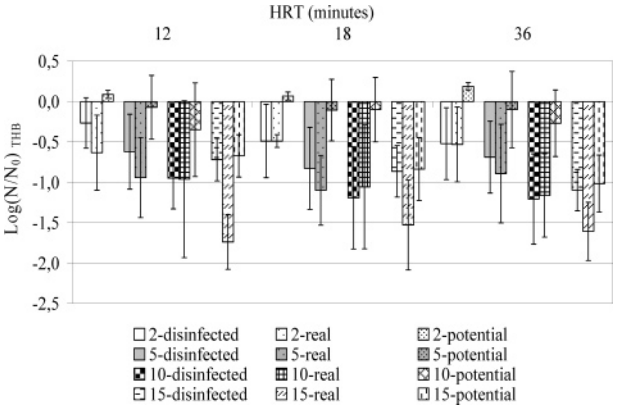

FIGURE 4. Mean log-reduction of THB, with the 95% confidence interval, in PAA disinfected effluent (disinfected), quenched PAA disinfected effluent after 5 h (potential), and PAA disinfected effluent after 5 h (real) as a function of disinfection contact time and PAA dose (values, expressed as mg/L, are reported in the legend). *N* stands for N_d for disinfected samples and N_r for real and potential re-growth samples.

PAA doses is related to the different disinfection efficiency (in fact, for the latter ones, $N_0 \gg N_d$).

DR values at a given re-growth time (t_r), for a fixed combination dose-contact time, appear to be almost comparable. This is due to the the following:

(1) PAA residual concentration depletion (see Figure 2) at the end of the first 5 h interval, for the lowest PAA doses, during which, in any case, no re-growth took place;

(2) Complete removal of disinfection surviving bacteria in the first 5 h interval, for the higher PAA doses.

Similar results were obtained for *E. coli*, even though DR values were lower (DR = -2×10^{-1} to -5×10^{-2} at 2 mg/L; DR = -2×10^{-3} to -2×10^{-4} at higher doses).

Therefore, for coliform group bacteria, the overall effect of PAA, despite the possible damage repair and the released acetic acid, is a long-lasting disinfection action.

To summarize, experimental results showed no appreciable re-growth for coliform bacteria for any of the combination dose-contact time tested, up to 29 h from the end of the disinfection treatment.

Total Heterotrophic Bacteria. For the THB group, no quality standard exists for discharge or reuse, but those bacteria could still be of some interest, both from a practical point of view (clogging of pumps and distribution systems due to the development of biofilms) and from the epidemiological point of view. Indeed coliform group bacteria do not represent the whole of infective risks, while various potential pathogens are included in total heterotrophic bacteria (i.e., *Pseudomonas aeruginosa*, *Streptococcus spp.*, etc.).

Data about potential and real re-growth for THB, enumerated by PCA, are shown in Figure 4. For coliform bacteria, when residual active PAA was present, microbial inactivation processes went on after the end of the selected contact time. Depending on PAA initial dose and contact time (HRT), log-reduction increased up to 1 unit with respect to disinfection. On the other hand, when residual PAA was quenched, Log N_r/N_0 reached positive values. For the lowest PAA dose (D = 2 mg/L) N_r was even bigger than N_0 (Log $N_r/N_0 > 0$). The reported data were highly variable, as shown by the large 95% confidence intervals, but this is quite a typical situation, due to the high number of different species and strains included in the THB group.

Mean values of Log(N_r/N_0) for THB, enumerated by PCA, are reported in Figure 5 for different re-growth times t_r (for residual PAA see Figure 2).

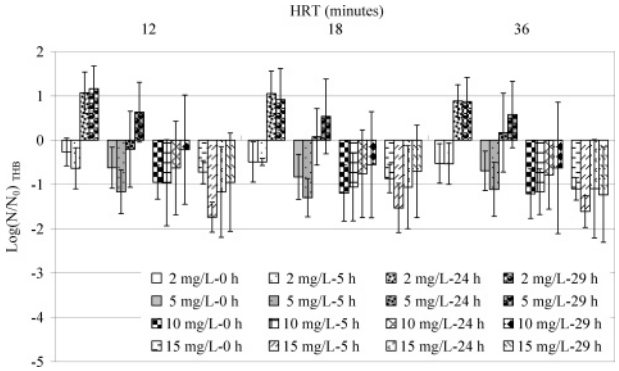

FIGURE 5. Mean log-reduction of THB, with the 95% confidence interval in samples at different re-growth time as a function of disinfection contact time (the legend gives the pairs of data: PAA dose−re-growth time). *N* stands for N_d for disinfected samples and N_r for real and potential re-growth samples.

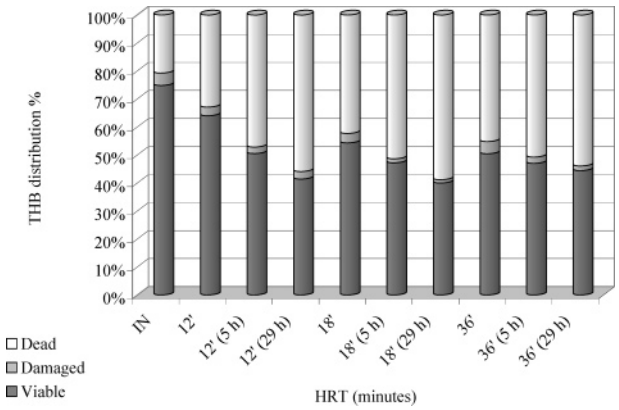

FIGURE 6. Percentage distribution of viable, damaged and dead THB obtained by cytometry, after selected contact times, and 5 and 29 h after sample collection operating at 5 mg PAA/L dose.

Experimental data highlighted a behavior depending on PAA initial doses and, above all, t_r . Re-growth was strictly related to the amount of residual PAA: for the lowest dose (2 mg/L), re-growth was observed starting from 24 h after disinfection and N_r became even higher than N_0 (about 1 order of magnitude) after 29 h. Re-growth was delayed for 5 mg/L and was not significant for the highest doses, even though N_r increased with respect to N_d. As shown in both Figures 4 and 5, disinfection efficiency of PAA against THB was limited (1 log-unit), so that after disinfection many bacteria were still intact; this fact in combination with the absence of residual PAA for a long time probably made the role of available acetic acid more significant than that of the death of damaged bacteria. Moreover, the variability of the trend observed may be also due to the strongly heterogeneous composition of THB.

The trend observed by cytometry was the opposite of the PCA based experiment. Cytometry used to enumerate total heterotrophic bacteria, showed the absence of microbial re-growth and a slight decrease of viable bacteria with increasing disinfection contact time and increasing post-disinfection contact time (Figure 6). The percentage of damaged bacteria was always very limited (<5%) and remained almost steady, as in the filtered water before disinfection. This confirms that the damage caused to microbial cells by PAA is permanent and causes their death.

For the lowest PAA doses, a re-growth trend for THB can be observed from PCA data, while the number of intact bacteria enumerated by cytometry decreases after the selected re-growth interval. It is likely that the stress deriving from low-dose disinfection prevents cells from forming

colonies when samples are collected immediately after treatment, while cells are able to recover from stress after a few hours. That temporary inhibiting effect is not detectable by cytometry as cytometry enumerates cells directly and differentiates between viable, damaged, and dead cells.

The number of microorganisms enumerated by cytometry was always significantly higher (1−2 orders of magnitude) than that obtained by plate count analyses. This difference depends on the specific properties of the different techniques: cytometry enumerates the single cells, while PCA allows the counting of colonies. This assumes that each cell generates a colony, which is often not the case, especially with environmental bacteria (*19*−*20*).

The absence of THB re-growth shown by cytometric analyses is particularly interesting when it is considered that room temperature (20−22°C) was certainly more favorable for environmental bacteria (THB) than for fecal coliform group bacteria, whose optimal range is over 40°C.

The two methods provide different results, especially about short-term disinfection efficiency. As a result of the present research, and taking into account re-growth, or rather, reactivation rates, data obtained by cytometry seem to be more reliable and give a more precautionary estimate of disinfection efficiency.

Acknowledgments

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