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COMPARATIVE STUDY ON THE EFFICIENCY OF PERACETIC ACID AND CHLORINE DIOXIDE AT LOW DOSES IN THE DISINFECTION OF URBAN WASTEWATERS

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Abstract: A comparison was made between the efficiency of low doses of peracetic acid (PAA: 1.5 mg/l) and chlorine dioxide (ClO_2: 1.5 and 2.0 mg/l) in the disinfection of secondary effluents of a wastewater treatment plant. Peracetic acid was seen to be more active than chlorine dioxide and less influenced by the organic content of the waste. Both PAA and ClO_2 (2.0 mg/l) lead to a higher reduction in total and faecal coliforms and *E. coli* than in phages (somatic coliphages and F-specific RNA bacteriophages) and enterococci. Detection of faecal coliforms and *E. coli* should therefore be accompanied by a search for these more resistant microorganisms when assessing the conformity of wastewater for irrigation use, or for discharge into surface waters. Coliphages are also considered suitable indicators of the presence of enteric viruses. Although the application of low doses of both disinfectants offers advantages in terms of costs and produces not significant quantities of byproducts, it is not sufficient to obtain wastewater suitable for irrigation according to the Italian norms (*E. coli* < 10/100 ml in 80% of samples and <100/100 ml in the remaining samples). Around 65% of the samples, however, presented concentrations of *E. coli* lower than the limit of 5,000/100 ml established by Italian norms for discharge into surface waters.

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INTRODUCTION

Water, once considered an inexhaustible commodity, is now regarded as a limited resource, not to be wasted. For this reason, wastewater reuse has been recommended in some countries as a possible source of water [1, 21, 28, 34, 41]. Although the use of untreated or inadequately treated wastewater may represent an important water resource for irrigation in semi-arid regions, as well as a source of nutrients for agricultural crops, this practice can entail risks for the health of both farmers and consumers and bring about changes in the ecosystems [1, 5, 33]. Moreover, the exportation of contaminated agricultural products can promote the diffusion of pathogenic microorganisms in areas where they are normally absent.

To ensure safe reuse of wastewater, guidelines have been prepared at an international level. The WHO established a limit of not more than 1,000 faecal coliforms per 100 ml for unrestricted irrigation of all crops [45], and the EPA set even more restrictive limits for total coliforms (TC ≤ 2.2/100 ml treated wastewater) and faecal coliforms (0 FC/100 ml) [5, 10]. Blumenthal *et al.* suggest less than 10^5 faecal coliforms/100 ml for restricted irrigation [4]. In Italy, the microbiological requirements for wastewater destined for irrigation are defined by the decree of the Ministry of the Environmental and Land Protection n. 185 of 2003 [8] and are repeated in the Legislative Decree n. 152 of 2006 [9]. The limits prescribed (*E. coli* < 10/100 ml for 80% of samples and a maximum value of 100/100 ml in the remaining samples) are much more restrictive than those

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set for wastewaters discharged directly into surface waters (*E. coli* < 5,000/100 ml) by the same Legislative Decree n. 152/2006.

Conventional wastewater treatments (primary and secondary) are able to remove a good deal of the BOD (Biochemical Oxygen Demand) and more than 90% of microorganisms responsible for enteric infections, but they are often insufficient to achieve the limits established by the norms [30]. Disinfection treatments are therefore necessary. Various products and/or techniques have been proposed for the disinfection of wastewaters. In the past, chlorine derivates were the most commonly used on account of their capacity to inactivate bacteria, viruses and protozoan cysts [43, 44]. However chlorination results in the formation of mutagenic/carcinogenic disinfection by-products (DBPs) (trihalomethans, haloacetic acids) deriving from the reaction of the chlorine with organic compounds [24, 31, 32].

Chlorine dioxide (ClO_2) is a strong disinfectant product able even to deactivate chlorine resistant parasitic pathogens such as *Cryptosporidium parvum* and to act in a wider range of pH [6]. Using chlorine dioxide, the formation of organohalogens (trihalomethanes and haloacetic acid) is lower when compared to the use of chlorine [11, 16]. This is attributed to the difference in oxidation reaction mechanisms, where chlorine dioxide reacts via free radical electrophilic abstraction versus oxidative substitution and addition for chlorine [3]. However, chlorine dioxide causes the formation of organic halides and chlorite and chlorate ions, which, if present in very high concentration in drinking waters, are suspected of being responsible for hematological alterations [15, 22, 37].

In order to avoid the drawbacks associated with the use of chlorine and derivates, alternative disinfecting agents have been proposed. A disinfectant adopted in recent years is peracetic acid, commercially available in a quaternary equilibrium mixture containing acetic acid, hydrogen peroxide, peracetic acid and water. It has strong oxidizing properties and is active against enteric bacteria, and to a lesser degree against viruses, phages, bacterial spores and protozoan cysts [25, 38, 39, 47]. When used in low doses, peracetic acid does not generate significant amounts of toxic or mutagenic by-products, or chemical residues in effluents [7, 25, 44].

This paper presents the results of a comparative study on the efficiency of peracetic acid (PAA) and chlorine dioxide (ClO_2) in the disinfection of secondary effluents in a large wastewater treatment plant. In particular, the investigation focused on whether the application of low doses of the two disinfectants, useful in reducing the costs of purification and limiting the formation of DBPs, is able to produce effluents that can be reused for irrigation. The action of the two disinfectants was tested for the usual bacterial indicators of faecal contamination (coliforms, *E. coli* and enterococci) as well as for somatic coliphages and F-RNA coliphages, which are considered markers of viral contamination. Tests were also carried out to detect the presence of *Escherichia coli* O157, an important human enteric pathogen, responsible for hemolytic uremic syndrome, and *E. coli* O157 phages.

METHODS

Facility. The study took place in a municipal wastewater plant located in Northern Italy, which treats a quantity of sewage amounting to approx. 1 million population equivalents. During the summer period, as foreseen by Italian norms, the effluents undergo primary (large and fine screening, sand and oil removal, and primary settling) and secondary treatment (biological treatment and secondary settling) and are then disinfected before being discharged into surface waters. The secondary effluent is treated contemporaneously with PAA and ClO_2. This is made possible by the presence of a disinfection basin with a chicane-type course involving two equal channels, which run down from the central distributor. The PAA, which is stored in a tank, is dosed by means of a diaphragm pump depending on the volume of the sewage, and is introduced through a pipe directly onto the surface of the wastewater. The chlorine dioxide is produced *in situ* through a direct reaction between HCl and $NaClO_2$. The solution, prediluted, is introduced into the secondary effluent using an appropriate system. The projected disinfection contact time was estimated on the basis of the overall size and shape of the basin and calculated to be approx. 18–20 min.

Sampling. Over a period of approximately six months (May–October 2006) 17 samplings were made, each involving 3 instant collections (at the time of greatest pollutant load): from secondary effluent (before disinfection), from secondary effluent after disinfection with PAA and from secondary effluent after disinfection with ClO_2. The investigation was carried out in two phases. During the first phase, made up of 8 samplings, 1.5 mg/l of PAA and 1.5 mg/l of ClO_2 were added; in the second phase (9 samplings) the dosage of ClO_2 was increased to 2.0 mg/l due to the poor level of efficiency shown by this disinfectant in the first phase.

The samples were taken from the exit point of the 2 channels of the disinfection basin before the treated effluents were mixed with disinfectants, that is approx. 200 m from the point of entry into the receiving body. A total of 51 samples were collected and subjected to physic-chemical and microbiological analysis. Any residue of disinfectant in the aliquots of wastewater due to undergo microbiological analysis were neutralized by adding 1 ml/l of 10% (w/v) sodium thiosulphate solution. The samples were kept under refrigeration during transport and analysed within 2 hours of collection.

Physical and chemical analysis. Temperature, pH, Total Suspended Solids (TSS), and Chemical Oxygen Demand

(COD) were measured using the techniques recommended in the Standard Methods [2].

For TSS measurement a mixed sample was filtered through a pre-weighed 0.45 micron membrane, which was dried to constant weight at 103 to 105°C. The suspended solids content, expressed as mg/l, was calculated by the increase in weight of the filter, relatively to the filtered volume.

For COD measurement a sample was refluxed in a strong acid solution (sulphuric acid) with a known excess of potassium dichromate. After digestion, the remaining potassium dichromate was titred with ferrous ammonium sulphate to determine the amount of dichromate consumed and the oxidable matter was calculated in terms of oxygen equivalent.

MICROBIOLOGICAL ANALYSIS

Total coliforms (TC), faecal coliforms (FC) and *E. coli* (EC). The multiple-tube fermentation technique was used [2]. In the presumptive test, appropriate sample dilution aliquots were inoculated in quintuple fermentation tubes containing Lauryl tryptose broth (Oxoid). After incubation at 35°C for 48 h all the positive tubes (showing growth and gas) were submitted to the confirmation phase for total coliforms in Brilliant green lactose bile broth (Oxoid) tubes incubated at 35°C for 48 h. For faecal coliforms EC broth (Oxoid) tubes, incubated in a water bath at 44°C for 24 h, were used for the confirmation test. For *E. coli* detection, from the presumptive fermentation tubes some loops were transferred into EC-MUG broth (Oxoid) tubes and maintained in a water bath at 44°C for 24 h. The presence of bright blue fluorescent light under long-wavelength UV lamp was considered positive.

***E. coli* O157.** Immunomagnetic separation method (IMS) was used, in accordance with ISO 16654 [19]. An aliquot of 25 ml was pre-enriched in 225 ml of Tryptic soy broth (Biolife), modified with novobiocine (20 mg/l). The samples were homogenized in Stomacher and incubated in a steam bath at 37°C, shaken/rotated at 150 rpm, for 18 h. Subsequently, 1 ml of pre-enrichment, as is and diluted 1:10, was transferred into 1.5 ml Eppendorf test-tubes and subjected to IMS with Dynabeads anti-*E. coli* O157 (Dynal, Oxoid). Two 50 ml aliquots of Dynabeads were seeded onto plates of Chromogenic *E. coli* O157 agar (Biolife) with the addition of CT selective supplement (cefixime and potassium tellurite) and incubated at 35-37°C for 18-24 h. Presumed colonies were confirmed by biochemical and serological tests. Biochemical identification was made using Enterotube II (BBL) and API 20E (bioMérieux), while serological testing made use of the *E. coli* O157 agglutination latex test (Oxoid).

Enterococci (ENT). The membrane filter technique was used [2]. Appropriate sample volumes were filtered through a 0.45 micron sterile membrane (Millipore). Filters were transferred to m-Enterococcus agar (Oxoid) in a Petri dish. After incubation at 35°C for 48 h, typical colonies were confirmed by growth on Bile esculine agar (Oxoid) at 35°C for 48 h and by growth on Brain-heart infusion broth (Oxoid) with 6.5% NaCl at 35°C for 48 h.

Somatic coliphages (SOMCPH), F-specific RNA bacteriophages (FRNAPH) and *E. coli* O157 phages (E.CPH). The double agar layer technique was used for the detection of somatic coliphages (ISO 10705-2) [18] and F-specific RNA bacteriophages (ISO 10705-1) [17]. For *E. coli* O157 phages, a modified version of the ISO 10705-1 method was used, involving growth medium without selective supplements. The sample was mixed with a small volume of semi-solid nutrient medium (Oxoid), with an appropriate aliquot of a 18-20 h culture of host strain (*E. coli* ATCC 700078 for somatic coliphages, *E. coli* ATCC 23631 for F-specific RNA bacteriophages and *E. coli* O157 ATCC 43888 for *E. coli* O157 phages) and plated on a solid Nutrient medium (Oxoid). After incubation at 36°C for 18 h, reading of plates for visible plaques was taken.

Presentation of results and statistical analysis. The values of the bacterial indicators and phages (somatic coliphages and F-RNA coliphages) were converted into $\log_{10}x$; the values of the *E. coli* O157 phages were converted into $\log_{10}(x+1)$. PAA and ClO_2 disinfection efficiency was assessed by determining microbial reductions, which is calculated as the decrease in $\log_{10}$ units between the number before and after disinfection treatment. Differences were considered significant as determined by ANOVA. A simple correlation test was used to evaluate the effect of secondary effluent characteristics on PAA and ClO_2 disinfection efficiency. $P \leq 0.05$ was considered significant.

All descriptive and statistical calculations were carried out using the StatView program (Abacus Concepts Inc., Berkley, CA, USA) on an Apple Macintosh computer.

RESULTS

Table 1 gives the physic-chemical characteristics of the effluents analyzed. In the second phase of the study, the secondary effluent showed higher values of temperature (mean: 24.6°C vs 21.8°C in the first phase) and a lower level of organic matter (mean COD value around 23% lower). The total suspended solids and pH were similar in both phases. After disinfection with both products no important variations were seen in the physic-chemical parameters. All samples, treated and non-treated, respected the COD limits of 125 mg/l set by the Legislative Decree 152/2006 for the discharge of effluents into surface waters from treatment plants with a potential of over 10,000 population equivalents. Only one sample, treated with PAA, exceeded the limit of 35 mg/l set by the same decree for TSS.

As far as the microbiological parameters are concerned (Tab. 2), the samples of secondary effluent showed concentrations of coliforms (total, faecal and *E. coli*) in the order

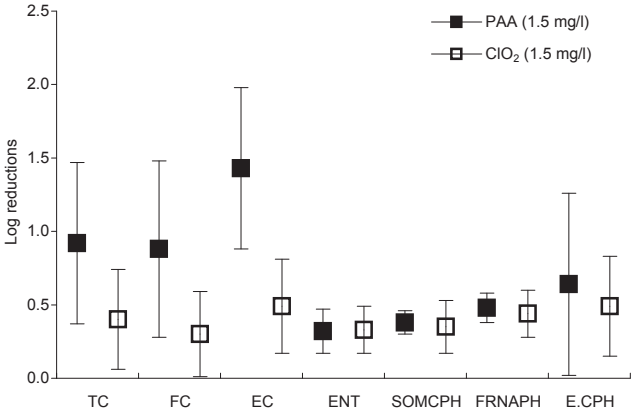

Figure 1. Average log reductions (bars showing 95% confidence intervals) of the different tested microorganisms (1st phase).

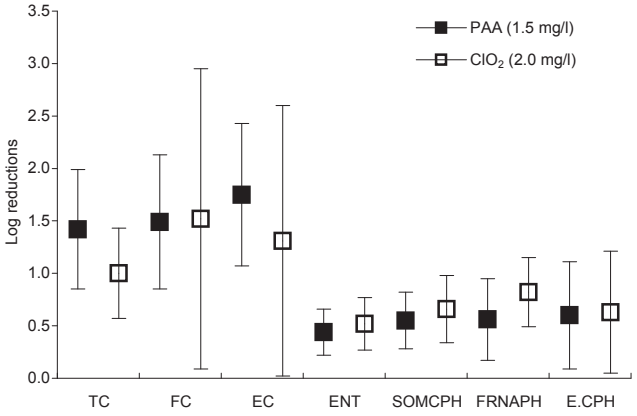

Figure 2. Average log reductions (bars showing 95% confidence intervals) of the different tested microorganisms (2nd phase).

of 5 log, enterococci and somatic coliphages in the order of 4 log and lower values of F-RNA bacteriophages (around 3 log). *E. coli* O157 was never detected; anti *E. coli* O157 phages were isolated from 82.3% of the samples. Table 2 also shows the residual microbiological contaminations after disinfection with PAA and ClO_2. Even after chemical treatment, in the absence of *E. coli* O157, anti-*E. coli* O157 phages were still found in 58.8% and 64.7% of samples disinfected respectively with PAA and ClO_2.

Figures 1 and 2 show the mean logarithmic reductions in the various tested microorganisms, respectively in the 2 phases of the investigation. PAA showed a greater efficiency against the coliforms (TC, FC, EC) compared to the other parameters; the reduction of *E. coli* was significantly higher than for enterococci and for all types of phages, in both the first (Fig. 1) and second phase (Fig. 2) (ANOVA, $p<0.05$). Moreover, in the second phase the peracetic acid produced on average higher levels of abatement, even though it was used in the same concentration as in the first phase (PAA: 1.5 mg/l). This can probably be explained by the variation in the composition of the wastewaters in the two periods in question, in particular the greater amount of organic matter in the first phase (higher values of COD) which may have interfered with the oxidising action, and the higher temperature which may have favoured the oxidative processes.

In the first phase of the study, the chlorine dioxide (ClO_2: 1.5 mg/l) brought about very small reductions (≤ 0.5 log), without any statistically significant differences between the microorganisms tested (Fig. 1). PAA was more effective than ClO_2 against the coliforms (TC, FC, EC), and in particular against *E. coli*, where the differences in the action of the two disinfectants are statistically significant (ANOVA, $p < 0.05$; Fig. 1); on the other hand, the action of the two products against enterococci and phages was almost the same. In the second phase (ClO_2: 2.0 mg/l), the highest dose of chlorine dioxide brought about an increase in the removal of coliforms (≥ 1 log per CT, CF, EC), and, to a lesser degree, of enterococci and phages. The differences in the relative abatement of the various microorganisms nevertheless remained not significant. A comparison between the effect of the two disinfectants on the

Table 1. Mean values, standard deviation and range of physic-chemical parameters before and after treatment with PAA and ClO_2.

	secondary effluent			PAA treated effluent			ClO_2 treated effluent		
	mean	SD	range	mean	SD	range	mean	SD	range
1st phase[a]									
Temperature (°C)	21.8	2.2	18.2–25.0	21.8	2.2	18.2–25.0	21.8	2.2	18.2–25.0
pH	6.67	0.12	6.50–6.81	6.72	0.17	6.54–7.08	6.68	0.12	6.54–6.91
TSS (mg/l)	17.1	10.0	5.1–32.0	18.9	8.2	1.2–29.9	17.5	8.7	5.3–27.0
COD (mg/l)	51.6	21.4	26.1–92.1	49.1	15.7	25.1–66.0	49.2	17.8	26.0–78.1
2nd phase[b]									
Temperature (°C)	24.6	1.9	22.2–27.0	24.6	1.9	22.0–26.9	24.6	1.9	22.0–26.9
pH	6.60	0.15	6.44–6.92	6.53	0.07	6.41–6.64	6.51	0.10	6.37–6.61
TSS (mg/l)	15.1	9.0	5.1–26.2	16.4	11.0	4.8–38.2	13.0	9.2	5.0–33.8
COD (mg/l)	39.8	16.8	10.9–60.0	43.0	18.7	15.2–78.0	40.1	20.3	11.9–80.3

[a] PAA 1.5 mg/l; ClO_2 1.5 mg/l, [b] PAA 1.5 mg/l; ClO_2 2.0 mg/l

Table 2. Mean values, standard deviation and range (log) of microbiological parameters before and after disinfection with PAA and ClO_2.

	secondary effluent			PAA treated effluent			ClO_2 treated effluent		
	mean	S.D.	range	mean	S.D.	range	mean	S.D.	range
1st phase[a]									
Total coliforms (MPN/100 ml)	5.65	0.88	4.38–6.73	4.73	1.12	3.23–6.21	5.25	1.15	3.80–6.73
Faecal coliforms (MPN/100 ml)	5.40	0.99	4.11–6.73	4.52	1.04	2.95–5.96	5.11	1.20	3.66–6.73
E. coli (MPN/100 ml)	5.11	0.96	3.69–6.24	3.69	1.13	2.52–5.54	4.62	1.51	2.85–6.54
Enterococci (CFU/100 ml)	4.61	0.83	3.26–5.80	4.29	0.89	3.11–5.56	4.28	0.94	3.00–5.60
Somatic coliphages (PFU/100 ml)	4.09	0.35	3.44–4.44	3.71	0.35	2.98–4.04	3.74	0.52	2.63–4.20
F + bacteriophages (PFU/100 ml)	3.67	0.34	3.13–4.08	3.19	0.39	2.57–3.73	3.23	0.60	2.18–3.85
E. coli O157 phages(PFU/100 ml)	1.58	0.94	0.00–2.48	0.94	1.12	0.00–2.35	1.09	1.05	0.00–2.24
2nd phase[b]									
Total coliforms (MPN/100 ml)	5.77	1.28	3.36–6.96	4.35	1.15	2.36–5.73	4.77	1.52	1.90–6.21
Faecal coliforms (MPN/100 ml)	5.57	1.21	3.36–6.73	4.08	1.30	2.34–5.73	4.05	2.00	0.21–5.96
E. coli (MPN/100 ml)	5.04	1.27	2.69–6.38	3.28	1.09	1.85–5.15	3.73	2.14	0.21–5.96
Enterococci (CFU/100 ml)	4.38	1.05	2.45–5.68	3.94	1.21	1.48–5.07	3.87	1.15	1.48–4.89
Somatic coliphages (PFU/100 ml)	4.35	0.41	3.32–4.66	3.79	0.66	2.30–4.51	3.69	0.65	2.30–4.22
F + bacteriophages (PFU/100 ml)	3.72	0.40	2.78–4.08	3.16	0.81	1.40–3.83	2.90	0.67	1.40–3.41
E. coli O157 phages (PFU/100 ml)	2.12	0.90	0.00–2.85	1.52	1.00	0.00–2.74	1.49	1.01	0.00–2.76

[a] PAA 1.5 mg/l; ClO_2 1.5 mg/l, [b] PAA 1.5 mg/l; ClO_2 2.0 mg/l

microbiological parameters in the second phase shows no statistically significant differences (Fig. 2).

The action of chlorine dioxide showed greater variability compared to peracetic acid, as demonstrated by the often high values of SD, probably due to the greater influence of the composition of the secondary effluent. Regression analysis showed that the efficiency of both disinfectants is negatively affected by the organic content of the secondary effluent, measured in terms of COD. However, when using PAA, the inverse correlations between COD and the reduction in microbiological parameters were statistically significant for enterococci (R^2=0.36, $p < 0.05$) and F-RNA phages (R^2=0.24, $p < 0.05$), while ClO_2 was statistically significant for total coliforms (R^2=0.59, $p < 0.001$), enterococci (R^2=0.28, $p < 0.05$) and coliphages (somatics: R^2=0.60, $p < 0.01$; F-RNA: R^2=0.51, $p < 0.01$). The process of disinfection was not, however, influenced by the other physic-chemical parameters, and the level of abatement was not correlated to the microbiological concentration in the secondary effluent.

According to the Italian norms, which set a limit of *E. coli* < 10/100 ml in 80% of samples and < 100/100 ml in the remaining 20% of samples, the secondary effluent is not suitable for agricultural use, even after treatment with PAA and ClO_2 (Tab. 3). As far as the WHO Guidelines are concerned, 17.6% of the samples disinfected with PAA and 33.3% of those treated with 2 mg/l of ClO_2 are compliant, while none of the samples meet the more restricted standards required by the EPA. With reference to the Legislative Decree 152/2006, which also regulates the discharge of wastewater into surface waters, the percentage of conformity is higher in the samples disinfected with peracetic acid than with chlorine dioxide (64.7% vs 33.3–37.5%) (Tab. 3).

DISCUSSION

The secondary effluent of the depuration plant under investigation presented a high variability of COD, TSS and microbiological parameters, due to the oscillation in the population served by the plant during the period of research (which included the holiday period), as well as the presence of a mixed sewage system that is affected by variations in atmospheric precipitation. Under the operative conditions in question, peracetic acid was seen to be more active than chlorine dioxide and less influenced by the variability in the composition of the waste. It is known from other studies that high levels of organic substances have a negative influence on the extent to which the microbiological indicators are reduced: a high COD compromises the performance of PAA [13] while chlorine dioxide is consumed during oxidation of the organic matter [29]. The levels of abatement achieved with chlorine dioxide were found to be inversely correlated to the concentrations of COD for more parameters than with peracetic acid. The suspended solids, contrary to the findings of other studies [14, 23, 25], do not appear to have influenced the efficacy of the disinfectants. Lazarova *et al.*, too, found that in the presence of levels of suspended solids comparable to those of the present study (11-40 mg/l), the impact on disinfection remained constant [26].

The microbiological indicators tested showed different levels of resistance to the chemical treatments in question.

Table 3. Compliance of samples with Italian limits and International Guidelines for the re-use in irrigation and discharge into surface waters.

	secondary effluent (17 samples)	PAA treated effluent (1.5 mg/l) (17 samples)	ClO_2 treated effluent (1.5 mg/l) (8 samples)	ClO_2 treated effluent (2.0 mgl/) (9 samples)
Irrigation reuse				
Italy, Ministry Decree 185/2003[a]				
% of samples <10 *E. coli*/100 ml	0	0	0	22.2
% of samples <100 *E. coli*/100 ml	0	11.8	0	11.1
WHO, 2006				
% of samples <1000 FC/100 ml	0	17.6	0	33.3
EPA, 2004				
% of samples with 0 FC/100 ml	0	0	0	0
Discharge into surface waters				
Italy, Legislative Decree 152/2006				
% of samples <5000 *E. coli*/100 ml	17.6	64.7	37.5	33.3

[a]samples are in compliance if *E. coli* <10/100 ml in 80% of samples and <100/100 ml in the remaining samples

At a dosage of 1.5 mg/l of peracetic acid and 2 mg/l of chlorine dioxide, the abatement of the phages, and above all of the enterococci, was much lower than that of the total and faecal coliforms, and *E. coli*. Chlorine dioxide, at a concentration of 1.5 mg/l, also showed low efficacy against the coliforms. The higher resistance of the coliphages and enterococci against disinfection has also been demonstrated in other studies [20, 23, 24, 25, 29, 35, 39, 43]. Since *E. coli* is the most sensitive microorganism to the two disinfectants, it would seem to be the least suitable for assessing the efficacy of a disinfectant process and the microbiological compliance of wastewaters. Therefore, in accordance with other authors, we propose that testing for this indicator should be accompanied by tests for more resistant microorganisms, such as enterococci and coliphages, the latter also being considered suitable indicators of the probable presence of enteric viruses [14, 27]. Gantzer *et al.*, in fact, found a significant correlation between the contamination of somatic coliphages and the presence of infectious enteroviruses ($p < 0.01$), and between the somatic coliphage concentration and the presence of the enterovirus genome ($p < 0.0001$) [12]. Steele and Odumeru reported that bacteria and protozoa tend to show the poorest survival outside a human host, whereas viruses and helminths can remain infective for months to years [40].

E. coli O157 was not detected in any of the samples examined, even when anti-*E. coli* O157 phages were found. Since the existence of these phages in the environment suggests the coexistence of its host strains in the same environment, it is possible that *E. coli* O157 was not isolated either because it was inactivated by the treatments, or because it is viable-but-non-culturable [42]. Some authors have reported that the disinfectants caused non-permanent and reversible damage, with the result that the indicators and human pathogens can reappear in wastewater some time after the disinfection – and may even regrow if nutrients are present [30, 36]. However, the possibility that *E. coli* O157 was masked by the abundant concomitant flora cannot be ruled out. In our previous studies carried out at the same plant, *E. coli* O157 was detected in only 2.8% of samples, above all in those presenting the lowest levels of bacterial indicators [46]. The difficulty encountered in isolating *E. coli* O157 from such complex and contaminated matrices as wastewaters suggests that it might be more feasible to test for anti-*E. coli* O157 phages (simpler and less expensive).

Due to the high concentration of bacterial and viral indicators, none of the samples of untreated secondary effluent respected the microbiological standards required by Italian law or by the WHO and EPA guidelines for reuse of wastewater for irrigation purposes. The disinfection of the secondary effluent with peracetic acid (1.5 mg/l) and chlorine dioxide (1.5 e 2.0 mg/l) was never sufficient to reduce the levels of *E. coli* to within the limits established in Italy, and only in a low percentage of samples was it found to be sufficient to meet the international requirements. The results are somewhat more satisfactory if considered in terms of the standards required by Italian law for wastewaters discharged into surface waters, especially as far as peracetic acid is concerned: around 65% of the samples presented concentrations of *E. coli* below the limit of 5,000/100 ml.

In conclusion, the following considerations can be made:

1) at the concentrations tested, peracetic acid appears preferable to chlorine dioxide in terms of depurative efficiency, cost and ease of management;

2) to assess the efficiency of disinfection in the treatment of municipal wastewaters, the detection of *E. coli* could be usefully accompanied by tests for more resistant microorganisms such as enterrococci and coliphages;

3) the application of low doses of both disinfectants, while offering advantages in terms of cost and the production of not significant quantities of by-products, is not sufficient to obtain wastewaters suitable for irrigation.

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