PERACETIC ACID AND SODIUM HYPOCHLORITE EFFECTIVENESS IN REDUCING RESISTANT STAGES OF MICROORGANISMS

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SUMMARY

A comparative study on the efficacy of peracetic acid and sodium hypochlorite in inactivating resistant stages of microorganisms such as *Cryptosporidium*, *Giardia* and *Cl. perfingens* was carried out. Furthermore the evaluation of the potential reciprocal correlation among the concentrations of the organisms was performed. The results obtained indicate that, at the same experimental conditions, peracetic acid and sodium hypochlorite have nearly similar reduction power against the resistant stages of *Giardia* and *Cl. perfringens*. Both the oxidants are instead less efficient in the abatement of *Cryptosporidium* oocysts. Findings have also confirmed our previous studies on the absence of association between *Clostridium* and the protozoa.

Key words: Clostridium perfringens, protozoa, peracetic acid, sodium hypochlorite, wastewater

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INTRODUCTION

Pathogens in water are generally considered a higher health risk than chemicals (1). Current drinking water disinfection practices provide the means to control most pathogenic microorganisms responsible for the major waterborne diseases. Most outbreaks in recent years have been caused by viruses and protozoa that are generally more resistant to chlorine-based disinfection than are pathogenic bacteria, the primary targets of concern in the past decades. Given the refractory nature of the protozoa *Giardia* and *Cryptosporidium* to standard water chlorination, significant effort has been expended to find new chemical agents, combinations, or conditions that could be used to effectively treat water. Furthermore, chlorine is also known to raise serious toxic effects on living organisms and several studies have demonstrated toxic, mutagenic and carcinogenic properties of its by-products (2, 3).

In the last years, peracetic acid has been introduced as disinfectant in agronomical and alimentary industries as well as in health sector, and only recently for wastewater disinfection (4, 5, 6). Its major advantages are the ease of implementing treatment, broad spectrum of activity even in the presence of heterogeneous organic matter and absence of persistent toxic or mutagenic residuals or by-products (7, 8).

Till now no data on the effects of peracetic acid on resistant stages, i.e. cysts and/or spores, of microorganisms in wastewater are available.

Taking in mind these issues, the current study was aimed at following: i) to monitor concentrations of the infective and resistant stages, oocysts and cysts, respectively, of *Cryptosporidium* and *Giardia*, in wastewater after a coarse screening and in the effluent after a secondary clarification in a municipal wastewater--treatment plant, ii) to estimate the efficacy of these treatments in removing *Cryptosporidium* oocysts, *Giardia* cysts and *Clostridium perfringens* spores, iii) to compare simultaneously the efficacy of two disinfectants (peracetic acid and sodium hypochlorite) in inactivating *Cryptosporidium* oocysts, *Giardia* cysts and *Cl. perfringens* spores during tertiary treatment, iv) to evaluate the potential reciprocal correlation among the concentrations of enteric protozoa oo/cysts and *Cl. perfringens* spores as putative indicator of their presence.

MATERIALS AND METHODS

Processing of sewage samples. Wastewater samples were obtained from a municipal plant located in Rome (Italy). The experimentation was partially carried out in a pilot plant installed in the municipal plant that uses a conventional wastewater-treatment system consisting of screening, primary clarification, aeration and biological oxidation through activated sludge, secondary clarification, and chlorination. The study was carried out on raw water (only subjected to a coarse screening), secondary effluent and two different tertiary effluents coming from alternative disinfection processes at the pilot plant.

Description of the pilot plant. The pilot plant consisted of a metallic structure subdivided in two parallel tanks. Each tank was employed for the contact between sewage and a disinfectant. Both the tanks were fed by the effluent from the secondary settler placed after the activated-sludge biological tank of the municipal plant. The effluent sucked by a submersible pump was introduced in the tanks by means of a pipe divided by a tee joint and a magnetic volume meter and a throttle valve were installed along each breach to obtain an independent control of the flow in the two tanks.

Residence times (t_R) of each tank were determined by injecting a tracer (6 l of a saturated solution of sodium chloride) into the sewage stream at the inlet of the pilot plant and by measuring the conductivity at the exit of the tanks. These measures were carried out at two different flow rates (2.0 and 8.0 l/s). The average ratio

Table 1. *Concentration*

Sample	Water after coarse screening			Water after secondary clarification			Reduction (%)		
N°	Cysts/l	Oocysts/l	Spores/l	Cysts/l	Oocysts/l	Spores/l	Cysts	Oocysts	Spores
1	1.5×10^4	1.2×10^3	1.4×10^6	1.5×10^3	5.0×10^2	6.0×10^4	90	58	96
2	3.1×10^3	$< 1.0\times10^0$	6.0×10^5	1.7×10^3	$< 1.0\times10^0$	3.1×10^4	45	–	95
3	3.0×10^4	6.0×10^2	1.4×10^5	4.3×10^3	1.5×10^2	2.7×10^4	86	75	81
4	5.7×10^3	4.5×10^2	3.0×10^5	6.2×10^2	2.1×10^2	7.3×10^4	93	53	76
5	2.7×10^3	3.0×10^3	n.d.	1.4×10^3	1.3×10^3	n.d.	48	57	–
6	1.2×10^4	1.0×10^3	2.5×10^5	3.0×10^2	3.9×10^2	1.3×10^4	98	61	95
7	2.5×10^4	1.0×10^3	2.0×10^4	3.1×10^3	4.3×10^2	1.4×10^3	88	57	93
8	3.5×10^4	8.0×10^2	5.0×10^5	2.1×10^3	2.2×10^2	8.0×10^3	94	64	98
9	2.5×10^4	$< 1.0\times10^0$	1.6×10^6	2.5×10^3	$< 1.0\times10^0$	n.d.	90	–	–

n.d.: not determined

between t_R and the time necessary to fill each tank was 0.78 for both contact chambers. The value of the actual contact time at varying of the flow rate was calculated by multiplying the average ratio by geometric volume of the fluid and the volumetric flow rate measured during each disinfection test.

Disinfection. Sodium hypochlorite (HYP) and peracetic acid (PAA) were tested in parallel during the tertiary treatment of the secondary effluent. The experiments were performed in the following conditions: disinfectant concentrations ranging from 2.0 to 4.5 mg/l, contact time of 20–30 min, pH 7 and temperature of 20 °C. Disinfectants were introduced in the respective tanks by two laboratory-made pneumatic pumps. The concentration of the disinfectants was determined daily before starting tests. Iodometric procedures were employed to measure levels of free chlorine and peroxiacid in sodium-hypochlorite and peracetic acid solutions, respectively. Free iodine liberated from KI by HYP or PAA was titrated against standardised $Na_2S_2O_3$ solution (0.1N). The interference caused by H_2O_2 in the determination of PAA titre was eliminated by adding some drops of catalase. Other analytical procedures were performed according to the methods described in the literature (9, 10).

***Cryptosporidium* and *Giardia* oo/cysts detection.** The detection was carried out on adequate volumes of the following samples:

i) raw water collected after a coarse screening;
ii) secondary effluent (water coming from secondary clarification, before entering the pilot plant);
iii) water running off the pilot plant after the treatment with sodium hypochlorite;
iv) water running off the pilot plant after the treatment with peracetic acid.

A total of 32 samples were collected in plastic tanks. Sodium thiosulfate (10 mg/l) and catalase (0.1 mg/l) were added to disinfected samples to inactivate any residual biocide. Cellulose-acetate membrane filters (Millipore, USA) were utilized to entrap *Cryptosporidium* oocysts and *Giardia* cysts from water samples and processed following the membrane filter dissolution method, according to Aldom and Chagla (11) and Graczyk and coll. (12). Sheather's sucrose density centrifugation was used to separate cysts and oocysts from suspended matter in the pellet (13). Parasites were detected by direct immunofluorescence with anti-*Giardia* and anti-*Cryptosporidium* monoclonal antibodies (Meridian Diagnostics, USA) taking into consideration morphology, size and color of the particles with respect to a positive standard. The presumed inactivation of cysts and oocysts was evaluated through the inspection of internal structures by contrast phase microscopy.

***Clostridium perfringens* spores detection.** Samples were pasteurized by heating to 75 °C for 15 min; spores were enumerated on OPSP (Perfringens Agar Base, Oxoid, UK) after incubation under anaerobic condition for 48 h. Black colonies were confirmed by the miniaturised system API 20A (Biomérieux, France).

Statistical analysis. Results were analysed with the statistical package SPSS v. 10.0.

RESULTS

In Table 1 the concentrations of *Giardia* cysts, *Cryptosporidium* oocysts and *Cl. perfringens* spores in water after the coarse screening and in the effluent after the secondary clarification are reported. The percentage of reduction was also calculated. As it was expected higher values were observed for *Cl. perfringens* spores, followed by *Giardia* cysts and *Cryptosporidium* oocysts that, contemporaneously, showed the highest variability. Their mean reduction after the clarification treatment was 81%, 61% and 91%, respectively. The extent of reduction was statistically significant for *Giardia* cysts ($p < 0.01$) and *Cl. perfringens* spores ($p < 0.01$).

In both the kind of water, no significative reciprocal correlation was observed among the three parameters.

Table 2 shows the reduction in the target organisms caused by the two alternative disinfection procedures. At the same c·t values, expressed as mg·min/l, sodium hypochlorite and peracetic acid exhibited nearly similar trends, with more uniform reduction values of oocysts and spores. The t-test, calculated with respect to the microorganism densities at c·t of 120 mg·min/l, has demonstrated that the concentration of *Giardia* cysts and *Cryptosporidium*

Table 2. *Reduction of Giardia cysts, Cryptosporidium oocysts and Cl. perfringens spores after the two different disinfection processes at the pilot plant*

	Water treated with sodium hypochlorite			Water treated with peracetic acid		
Sample N°	**2**	**2**	**3**	**2**	**2**	**3**
C . t (mg . min/l)	**60**	**90**	**120**	**60**	**90**	**120**
Reduction (%) of *Giardia* cysts	94.0	97.0	98.0	92.8	96.5	91.6
Reduction (%) of *Cryptosporidium* oocysts	73.0	74.0	68.4	71.0	75.0	74.5
Reduction (%) of *Cl. perfringens* spores	99.3	99.8	n.d.	97.4	99.7	n.d.

n.d.: not determined

Table 3. *Survival of Giardia cysts, Cryptosporidium oocysts and Cl. perfringens spores in the post-disinfected effluents*

	Mean percentage of surviving organisms*					
	Sodium hypochlorite treatment			Peracetic acid treatment		
C . t (mg . min/l)	**60**	**90**	**120**	**60**	**90**	**120**
N°**	**2**	**2**	**3**	**2**	**2**	**3**
Giardia cysts	49	43	48	57	50	78
Cryptosporidium oocysts	75	73	77	80	76	71
Cl. perfringens spores	3	7.5	n.d.	28	31	n.d.

*Post-disinfected /pre-disinfected densities (×100); **N°: number of paired pre- and post-disinfected samples
n.d.: not determined

oocysts in the effluents treated with sodium hypochlorite was not significantly different from that present in the effluent treated with peracetic acid.

Values of the survival mean percentage of protozoan oo/cysts and *Cl. perfringens* spores in the post-disinfected effluent in respect to their densities in the pre-disinfected effluent are reported in Table 3. Different survival values were calculated for *Cl. perfringens* with respect to *Giardia* and *Cryptosporidium*. In fact, spores appeared to be more affected by both the disinfectants than *Giardia* and *Cryptosporidium* and much more by sodium hypochlorite than peracetic acid. The total mean percentage of spore inactivation was 95% and 71%, respectively, when sodium hypochlorite and peracetic acid were used. For *Giardia* cysts and *Cryptosporidium* oocysts, less influenced by both the disinfectants, the calculated values reached the 53% and 38% and the 25% and 24%, respectively.

DISCUSSION AND CONCLUSIONS

Data collected during this investigation indicate that high numbers of oocysts, cysts and spores were detected in water treated at the municipal wastewater-treatment plant. All samples were positive for cysts and spores, whereas the 22% of the samples gave negative results for oocysts. These findings confirm other studies conducted in Italy where *Giardia* is detected more frequently in wastewater and at higher concentrations than *Cryptosporidium* (14). The lower *Cryptosporidium* density in waste water could be due to its probable actual lower occurrence in water in general but also due to methodological difficulties in oocysts detection caused by its small size. In fact, taking into consideration the recovery efficiency of the method applied to raw water that, for *Cryptosporidium* oocysts, corresponds to the 25–40%, it is presumable an underestimation of its concentrations.

Variable values of reduction of the resistant stages of both the protozoa were calculated according to the concentrations obtained after the secondary clarification. Values ranging from the 45% to 98% for *Giardia* and from the 53% to 75% for *Cryptosporidium* were recorded. They could be attributed to the changeable turbidity values measured in the analyzed waters (4÷18 NTU). In fact, higher water turbidity values can interfere with the protozoa recovery (15). Besides, while the extent of reduction was statistically significative for *Giardia* and *Cl. perfringens*, it was not for *Cryptosporidium*. Once more, it could be ascribed to the larger size of cysts and spores, probably more affected by the sedimentation forces to which the effluent was subjected during the secondary clarification treatment.

The results obtained at the pilot plant indicate that, under the same experimental conditions, peracetic acid and sodium hypochlorite have nearly similar reduction power against the resistant stages of *Giardia* and *Cl. perfringens*. Both the oxidants are less efficient, and equivalent, in the abatement of *Cryptosporidium* oocysts, also at the highest dose (concentration 4.5 mg/l, contact time 30 min), thus confirming their greater resistance to biocides.

As far as the possible role of *Cl. perfringens* spores as indicator of occurrence of protozoan oo/cysts in treated water is concerned, our results evidenced the absence of association between *Clostridium* and the protozoa. This lack of consistent relationship in the examined water strengthen once again data previously find by the authors (16): the numbers of *Cl. perfringens* cannot be used to assess the risk of *Cryptosporidium* and *Giardia*.

Investigations of commonly used water treatment technologies indicate that both the protozoa are highly resistant to water treatment processes (17). Physical methods (microfiltration and UV irradiation) or sequential treatment using different disinfection agents have been found to be useful in certain circumstances (18). For this reason, in relation to the preliminary results obtained in this investigation, further studies are planned to establish the effective doses of peracetic acid necessary to give satisfactory reduction in protozoan numbers in sewage effluents under operational conditions and to identify its potential by-products.

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HIV VACCINE GLOBAL PARTNERS STRENGTHEN COLLABORATION TO SPEED UP PROGRESS

Geneva – As new developments in the search for an HIV vaccine take place, vaccine researchers from around the world are joining forces to accelerate progress towards an effective and safe HIV vaccine, with the full and equal involvement of countries most affected by the AIDS epidemic.

"With so many HIV vaccine clinical trials testing novel products ongoing and planned by a wide variety of investigators, it is time to intensify global collaboration. Lessons learned must benefit all working in this challenging, but advancing, field," said Dr Marie-Paule Kieny, Director, Initiative for Vaccine Research, World Health Organization (WHO).

About 50 experts from developing and industrialized countries presented and discussed their HIV vaccine research and development efforts at the first WHO-UNAIDS Meeting of Global Partners Promoting HIV Vaccine Research and Development, which took place in Montreux, Switzerland, on 2–3 February. The participating vaccine experts are from governments, academia, industry, public-private partnerships and non-governmental organizations throughout the world.

Recent progress in the HIV vaccine area includes the completion of several phase I and II trials of candidate vaccines. The publication last month of the Global HIV/AIDS Vaccine Enterprise Scientific Strategic Plan has also set a number of important milestones to be reached by all global partners.

But challenges remain. They include the need to increase clinical trial capacity worldwide and conduct trials at multiple sites against different globally prevalent HIV strains in populations with different transmission patterns; the appropriate use of trial sites for other HIV preventive research; the interface between HIV vaccine trials and increased access to anti-retroviral treatment; and the need to ensure that the most appropriate candidate vaccines are tested at the most appropriate sites regardless of who developed the product or strengthened the site.

"Overcoming these challenges will require intense international collaboration and coordination," said Dr Saladin Osmanov, Acting Coordinator, WHO-UNAIDS HIV Vaccine Initiative.

Twenty-five million people in sub-Saharan Africa are currently living with HIV, accounting for over 65% of all infections worldwide. Developing countries must be involved as equal partners in the development of HIV vaccines. An increasing number of trials are planned in African countries. This has not always been the case. Although the first clinical trial of an HIV vaccine took place in 1987 and more than 70 phase I HIV vaccine trials have since taken place, by 2003 only four phase I/II trials had been conducted on the African continent.

"Africa must participate in HIV vaccine development," said Dr. Pascoal Mocumbi, High Representative, European and Developing Countries Clinical Trials Partnership and former Prime Minister of Mozambique. He added that the majority of African countries are more focused on disease control and very few have provisions for HIV vaccine research and development in their national AIDS programmes.

It is important to conduct vaccine trials in developing countries because the genetic variability of HIV may require testing of vaccine candidates in different areas of the world, where different strains are prevalent. It may also be necessary to evaluate how different infection routes, cofactors for HIV transmission, such as other sexually transmitted infections, and host genetic backgrounds influence vaccine-induced protection. Finally, licensing of a successful vaccine by regulatory bodies may require prior trials in countries with similar epidemiological settings.

The WHO-UNAIDS supported African AIDS Vaccine Programme (AAVP), established in 2000, is a network of African experts interacting with global partners and working together to promote and facilitate HIV vaccine research and evaluation in Africa, so that appropriate vaccines are developed and made