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Genotoxicity of the disinfection by-products resulting from peracetic acid- or hypochlorite-disinfected sewage wastewater

R. Crebelli[a], L. Conti[a], S. Monarca[b], D. Feretti[b], I. Zerbini[b], C. Zani[b], E. Veschetti[c,*], D. Cutilli[c], M. Ottaviani[c]

[a]*Laboratory of Comparative Toxicology and Ecotoxicology, Istituto Superiore di Sanità, Rome, Italy*
[b]*Department of Experimental and Applied Medicine, Hygiene Section, University of Brescia, Brescia, Italy*
[c]*Laboratory of Environmental Hygiene, Istituto Superiore di Sanità, NCFQRA, Viale Regina Elena 299, Rome 00161, Italy*

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Abstract

Wastewater disinfection is routinely carried out to prevent the spread of human pathogens present in wastewater effluents. To this aim, chemical and physical treatments are applied to the effluents before their emission in water bodies. In this study, the influence of two widely used disinfectants, peracetic acid (PAA) and sodium hypochlorite (NaClO), on the formation of mutagenic by-products was investigated. Wastewater samples were collected before and after disinfection, in winter and in summer, at a pilot plant installed in a municipal wastewater-treatment plant. Samples were adsorbed using silica C_{18} cartridges and the concentrates were tested for mutagenicity in the *Salmonella typhimurium* reversion test with strains TA98 and TA100. Non-concentrated water samples were tested with two plant genotoxicity assays (the *Allium cepa* root anaphase aberration test and the *Tradescantia*/micronucleus test). Mutagenicity assays in bacteria and in *Tradescantia* showed borderline mutagenicity in some of the wastewater samples, independent of the disinfection procedure applied. Negative results were obtained in the *A. cepa* anaphase aberration test. These results indicate that, in the conditions applied, wastewater disinfection with PAA and NaClO does not lead to the formation of significant amounts of genotoxic by-products.
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1. Introduction

Chlorine disinfection is widely used in the tertiary treatment of urban wastewaters, with the aim of reducing microbial contamination and preventing the spread of pathogens into the environment. However, chlorine can react with natural organic substances (humic and fulvic acids) present in surface waters, giving rise to numerous volatile and non-volatile disinfection by-products (DBP) with mutagenic and/or carcinogenic activity (Rook, 1974; Daniel et al., 1993; Glaze et al., 1993; DeMarini et al., 1995; Meier et al., 1996). It is therefore necessary to search disinfectants alternative to chlorine which are effective against microbial contamination of wastewater, and concurrently reduce DBP.

Peracetic acid (PAA) is a proposed alternative to chlorine for the disinfection of urban effluents. PAA was

*Corresponding author. Tel.: +39 06 4990 3063; fax: +39 06 4938 7083.
E-mail address: repacqua@iss.it (E. Veschetti).

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found to be effective against bacteria and viruses present in urban wastewater, and slightly affected by the presence of organic matter in the medium (Baldry and French, 1989; Baldry et al., 1991, 1995; Lefevre et al., 1992). Disinfection of surface water used for human consumption with PAA has recently been shown to produce only carboxylic acids, devoid of mutagenic properties (Monarca et al., 2002). However, no investigation on the influence of PAA on the formation of DBP from wastewater disinfection has been carried out.

Short-term mutagenicity assays have been widely used to assess the formation of genotoxic DBP from wastewater disinfection. To this aim, both bacterial mutagenicity tests (Saxena and Schwartz, 1979; Meier and Bishop, 1985; Meier et al., 1987; Monarca et al., 2000) and plant genotoxicity assays (Rank and Nielsen, 1994; Monarca et al., 2000) have successfully been used to evaluate the genotoxicity of DBP. In this work, the formation of DBP in municipal wastewater samples disinfected with PAA or chlorine (NaClO) has been investigated with in vitro bacterial reversion assays and with plant genotoxicity tests.

2. Materials and methods

2.1. Description of the plant

The experimentation was carried out in a pilot plant installed at a municipal wastewater-treatment plant located in Rome (Italy). The municipal plant uses a conventional sewage-treatment system based on screening, primary clarification, aeration and biological oxidation through activated sludge, secondary clarification, and chlorination. Detailed information on the structure of the apparatus has been reported elsewhere (Veschetti et al., 2003). Residence times (t_R) of each tank were determined by injecting rapidly 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 electrical conductivity at the exit of the tanks. Instantaneous flow rates of the tracer added to the sewage were also recorded during the determination of the conductivity profiles.

2.2. Wastewater treatments

NaClO and PAA solutions containing 5% or 15% of technical-grade disinfectant were supplied by Zarrelli (Rome, Italy) and Solvay Interox (Brussels, Belgium), respectively. Their actual concentration was determined daily by iodometric analyses before starting tests. Treatment conditions (final concentration of PAA or NaClO in wastewater and time of contact) were as follows:

- First treatment: 4 mg/l for 37 min;
- Second treatment: 4.1 mg/l for 26 min;
- Third treatment: 2 mg/l for 37 min;
- Fourth treatment: 4 mg/l for 26 min.

At the end of treatment period, both untreated and the disinfected wastewater samples were added with ferrous sulfate ($FeSO_4$) to eliminate free disinfectants. Unconcentrated water samples, with and without $FeSO_4$, were tested in toto in the plant genotoxicity tests. Samples tested in bacterial assays were concentrated as described below.

2.3. Concentration of wastewater samples

Wastewater samples collected before and after disinfection treatments were added with $FeSO_4$, then passed on filter paper (Whatman 5) to eliminate the suspended solids, acidified with hydrochloric acid at pH 2–2.5, and passed on trifunctional silica C_{18} cartridges (Sep-Pak Plus tC_{18} Environmental Cartridges, Waters Chromatography) according to US EPA 525.2 method (US Environmental Protection Agency, 1995). The cartridges had previously been washed with 5 ml of ethyl acetate, 5 ml of dichloromethane, 10 ml of methanol and 10 ml of distilled water. Two litres of wastewater samples were adsorbed on each cartridge, which were then dried under a flow of nitrogen and eluted with 5 ml ethyl acetate and 5 ml dichloromethane. The eluates were reduced to a small volume by means of a rotating vacuum evaporator, mixed and dried under nitrogen flow. The dry residue was dissolved in dimethylsulfoxide (DMSO) and stored in the dark at −20 °C.

2.4. Chemical analyses

Duplicate wastewater samples were collected in 500-ml glass bottles at the inlet and outlet of the pilot plant. Sampling operations were repeated three times at each programmed experimental condition to determine the reproducibility of the results. Sodium sulfite (0.50 g) was introduced in all bottles containing the disinfected wastewater in order to reduce the residues of the oxidizing agents. One sample was acidified with 2 ml of concentrated sulfuric acid (96%). Collected samples were stored at approximately 4 °C and subsequently analyzed.

Total organic carbon (TOC), adsorbable organic halogens (AOX) and ammonia were determined in the acidified samples, while nitrite, nitrate and total suspended solids (TSS) were dosed in the remaining samples. All these analytic procedures were carried out

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