Inactivation of bacteria and helminth in wastewater treatment plant effluent using oxidation processes
Regiane Aparecida Guadagnini, Luciana Urbano dos Santos, Regina Maura Bueno Franco and José Roberto Guimarães

ABSTRACT
The contamination of bodies of water by raw and even treated sewage is worrying because pathogens that affect public health and the environment are not fully eliminated in wastewater treatment systems. The disinfection step is an important barrier to adopt to reduce this contamination. However, widely used disinfectants such as chlorine do not guarantee the inactivation of resistant organisms such as spore-forming bacteria and helminth eggs. This study evaluated the effectiveness of processes of peroxidation (H₂O₂), ultraviolet radiation (UV) and peroxidation assisted by ultraviolet radiation (H₂O₂/UV) in terms of reduction and inactivation of total coliform bacteria, *Escherichia coli*, helminth eggs and larvae present in a treated sewage. Doses of UV radiation of 70 mJ cm⁻² and hydrogen peroxide concentration of 30 mg L⁻¹ were used. The number of bacteria reduced after UV and H₂O₂/UV processes was 3 and 4 log, respectively. An average reduction of 59% in the number of eggs was verified when using H₂O₂, UV, and H₂O₂/UV processes. Helminth larvae were reduced by 24% after H₂O₂ and UV; the process H₂O₂/UV did not reduce the number of larvae. Statistically significant differences between the processes for both organisms were not observed.

Key words | advanced oxidation process, disinfection, helminth eggs

INTRODUCTION
In recent decades, the world's population has increased significantly with consequent growth in waste generation and demand for water that is suitable for consumption. Chemical compounds and microorganisms emerging and re-emerging in bodies of water increase the cost of making water drinkable. The World Health Organization (WHO 2006) estimated that 1.1 billion people did not have access to drinking water and 2.6 billion people lacked adequate sanitation. This scenario leads to a serious public health problem because water quality affects people's health, especially children. The concern with respect to drinking water quality is growing because many worldwide outbreaks of waterborne protozoa were registered between January 2004 and December 2010 (Baldursson & Karanis 2011). Among possible waterborne pathogens, helminths were listed as being responsible for 6% of infections (WHO 2005). The helminth was also the most common of the human parasites, causing a series of health complications, such as chronic infections and reduction in productivity and decline in physical and cognitive development in children.

These parasites affect over 2 billion people, of which 1 billion people are infected with *Ascaris lumbricoides*, a parasite of global distribution. Ascariasis is a neglected disease frequently associated with another species of helminth, *Trichuris trichiura*, and other diseases such as malaria and HIV (Scott 2008; Holland 2009). Infection has been reported in 5-month-old children and the prevalence and intensity increase with age (Scott 2008).

Due to its high prevalence and close association with poor infrastructure, helminths are used as a social indicator of a country. The lack of sanitation and behavior such as poor hygiene habits are the forms of disease transmission.

Raw and treated sewage are among the main causes of contamination of water sources. They may contain the infectious forms of helminths and various other pathogenic species (Cantusio Neto et al. 2006). In some Brazilian states, legislation establishes parameters for the quality of
treated wastewater which depend on the quality level of the receptor. An 80% reduction in biochemical oxygen demand (BOD) is required. Only bacteria of the coliform group are evaluated because they are considered a major environmental indicator of fecal contamination. Escherichia coli is the main thermotolerant coliform and the most efficient indicator of contamination by fecal matter, since it is restricted to the intestinal tract of warm-blooded animals (Soller et al. 2010). Parasites are much more resistant to chemical processes than bacteria, and resistant forms may remain infectious for several months in the environment. An aggravating factor is that parasitological assessment is not routinely performed in water and wastewater treatment plants. Therefore, the control of bacteria as an indicator of water quality cannot be used as an indicator of parasites.

The Atibaia River is the main water source for treatment in the city of Campinas in the state of São Paulo in southeastern Brazil. This river crosses a large metropolitan and industrialized area and receives a high load of fecal material discharged as domestic effluent as demonstrated by the mean values of thermotolerant coliforms of the order of 10^5 MPN/100 mL (Cantusio et al. 2010). Toxoplasma gondii and Cyclospora cayetanensis protozoa were recently responsible for a waterborne outbreak of disease in the southeast and south of Brazil (Zini et al. 2004; Moura et al. 2006). These records indicate the fragility of the sanitation system and the importance of the disinfection process as a barrier to reducing these pathogens.

The water chlorination process ensures safety in relation to viruses and bacteria, but does not ensure the inactivation of helminths (Mun et al. 2009) and there are few conclusive studies on inactivation of helmint eggs. For example, helmint eggs of the genus Ascaris are extremely resistant and survive numerous treatment conditions. Aladawi et al. (2006) evaluated a disinfection process using ultraviolet radiation and observed a 2 log inactivation of A. lumbricoides with a UV dose of 400 mJ cm^-2; however, in a study by Brownell & Nelson (2006), this inactivation level was not achieved using UV doses of up to 15,000 mJ cm^-2. Mun et al. (2009) reduced 2.5 log helmint eggs after 60 s (700 W) using microwave radiation. These variations observed in existing results confirm the need for studies on the effect of different water disinfection processes on the helminths.

Advanced oxidation processes (AOP) have a high oxidation potential mainly due to the generation of hydroxyl radicals (•OH) (E = 2.80 V). These free radicals are not selective and interact with a wide range of recalcitrant compounds and cell components. AOP are usually considered clean technologies; they can mineralize organic compounds, generating products that aren’t as harmful to health (Cordeiro et al. 2004; Cho & Yoon 2008). They have been shown to be effective at inactivating Clostridium perfringens and coliphages (Guimarães & Barretto 2005), Escherichia coli, Bacillus subtilis, MS2 virus (Mamane et al. 2007; Alrousan et al. 2009) and protozoa (Cho & Yoon 2008; Guimarães et al. submitted).

There are several combinations used in AOP to obtain the hydroxyl radical, among them ultraviolet light-assisted peroxidation (H_2O_2/UV). This has the advantage of a high oxidizing potential, and hydrogen peroxide is commercially available, thermally stable, and can be stored at the point of use (Domènec et al. 2004). Theoretically, a molecule of H_2O_2 produces two hydroxyl radicals (Equation (1)) when subjected to a source of energy sufficient to break the bond between the two oxygen atoms with quantum yield (Φ) of 0.98 at 254 nm:

\[ H_2O_2 + hv \rightarrow 2^*OH \]  
(1)

When in excess, the hydrogen peroxide can produce other types of reactions. They are competitive and can have the effect of reducing degradation efficiency (Domènec et al. 2004). Studies are needed to provide an optimum concentration of oxidant and UV radiation dose for the inactivation of different target species.

The aim of this study was to evaluate the efficiency of processes of peroxidation (H_2O_2), ultraviolet radiation (UV) and peroxidation assisted by ultraviolet radiation (H_2O_2/UV) in the reduction and inactivation of total coliform bacteria and Escherichia coli and helmint eggs and larvae present in a treated domestic sewage.

**MATERIALS AND METHODS**

The samples (n = 4 for bacteria and n = 5 for helminths) were collected at Samambaia Wastewater Treatment Plant (Samambaia WWTP) in Campinas, Brazil. The plant receives only domestic sewage and has the capacity to treat 150 L s^-1. Samambaia WWTP uses a conventional sewage treatment system based on activated sludge and secondary clarification.

In order to evaluate the efficiency of bacteria and helmint removal by the activated sludge process, two sampling points were selected: (1) influent and (2) effluent. To evaluate the oxidative process, five samples of the effluent were taken in jugs that had been previously
conditioned with 0.1% Tween 80 eluting solution. Samples were refrigerated at 10 °C and taken to the Laboratory of Oxidative Processes (LABPOX) for the tests and analysis. LABPOX is part of the Civil Engineering, Architecture and Urban Design School of the University of Campinas Department of Sanitation and Environment.

Disinfection process: reactor

The experimental system consisted of a photochemical reactor designed by Guimarães et al. (submitted): a 42.5 cm long borosilicate glass cylinder with an internal diameter of 3.8 cm and a gemicidal lamp (15 W, λmax = 254 nm, and 2.5 cm internal diameter) inserted in the center in direct contact with the sample. The internal volume of the internal reactor is 190 ± 0.05 mL, and the system also includes a reservoir (2 L) and a peristaltic pump to the solution in movement.

The assays were carried out using 70 mJ cm⁻² doses of UV radiation. Initial hydrogen peroxide concentration was 30 mg L⁻¹. The following conditions were used: (a) only in the presence of hydrogen peroxide (H₂O₂); (b) only in presence of ultraviolet radiation (UV); and (c) in the presence of hydrogen peroxide and ultraviolet radiation (H₂O₂/UV). The UV and peroxidation processes were used as a control to evaluate the synergistic effect of AOP.

The system was decontaminated before and after each test to remove possible residues and microorganisms that may be retained in the system. The experimental conditions and procedures proposed followed the protocols established by Guimarães et al. (submitted).

Bacterium and helminth detection and inactivation

Quanty-Tray®/2000 test (IDEXX Laboratories, Incorporation) based on IDEXX Enzymatic Defined Substrate Technology® was used to evaluate the inactivation of bacteria by oxidative processes. The test had a detection limit of 1 MPN/100 mL and maximum limit of 2,419.6 MPN/100 mL. The samples were incubated for 24 h at 35 °C.

Helminth analyses from processed or unprocessed wastewater samples were performed according to the Victoria & Galván (2003) protocol. One liter of effluent sample was centrifuged at 480 × g for 5 min in 50 mL centrifuge tubes. After removing the supernatant, the pellet was washed with distilled water and centrifuged at 480 × g for 5 min. After two washes, the pellet was resuspended by zinc flotation and centrifuged at 480 × g for 5 min. The supernatant was filtered through a 47 mm diameter 5.0 μm membrane. The membrane was submitted to sample elution by alternately scraping the membrane with a smooth-edged plastic loop and rinsing with elution solution. The final 1 mL pellet was transferred to a microcentrifuge tube after a homogenization protocol (Redlinger et al. 2002). Aliquots of 15 μL of the samples were tested with Trypan Blue (1%) for quantification and verification of possible egg inactivation.

Statistical analysis

Student’s t-test with a p-value < 0.05 was used to analyze the data.

RESULTS AND DISCUSSION

The evaluation of the disinfection processes’ efficiency was based on the inactivation and/or the number of microorganisms reduced. Total coliform (TC), E. coli (EC) bacteria, and helminth eggs and/or larvae were found in every influent and effluent sample. The TC quantification varies between samples (Table 1) with an average of 7.2 × 10⁷ MPN/100 mL in influent samples and 2.5 × 10⁵ MPN/100 mL in effluent samples. The EC quantification varies between samples (Table 2) with an average of 2.2 × 10⁷ MPN/100 mL in influent samples and 3.1 × 10⁴ MPN/100 mL in effluent samples.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Mean</th>
<th>SD</th>
<th>Max.</th>
<th>Min.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Influent</td>
<td>7.20 × 10⁷</td>
<td>1.90 × 10⁷</td>
<td>9.20 × 10⁷</td>
<td>4.90 × 10⁷</td>
</tr>
<tr>
<td>Effluent</td>
<td>2.45 × 10⁵</td>
<td>9.88 × 10⁴</td>
<td>3.70 × 10⁵</td>
<td>1.40 × 10⁵</td>
</tr>
<tr>
<td>Effluent + peroxidation</td>
<td>1.08 × 10⁵</td>
<td>2.39 × 10⁴</td>
<td>1.40 × 10⁵</td>
<td>8.30 × 10⁴</td>
</tr>
<tr>
<td>Effluent + UV radiation</td>
<td>6.20 × 10¹</td>
<td>4.97 × 10¹</td>
<td>1.10 × 10²</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Effluent + AOP</td>
<td>1.01 × 10¹</td>
<td>1.50 × 10¹</td>
<td>3.20 × 10¹</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Samples</th>
<th>Mean</th>
<th>SD</th>
<th>Max.</th>
<th>Min.</th>
</tr>
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<tbody>
<tr>
<td>Influent</td>
<td>223 × 10⁷</td>
<td>6.08 × 10⁶</td>
<td>2.10 × 10⁷</td>
<td>1.70 × 10⁷</td>
</tr>
<tr>
<td>Effluent</td>
<td>3.05 × 10⁴</td>
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<td>4.50 × 10⁴</td>
<td>2.30 × 10⁴</td>
</tr>
<tr>
<td>Effluent + peroxidation</td>
<td>5.55 × 10⁵</td>
<td>7.65 × 10⁴</td>
<td>1.70 × 10⁵</td>
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<td>Effluent + UV radiation</td>
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<tr>
<td>Effluent + AOP</td>
<td>2.03</td>
<td>2.49</td>
<td>5.1</td>
<td>&lt;1</td>
</tr>
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</table>
The activated sludge treatment system applied in the WWTP was seen to be responsible for a reduction of approximately 2 log of total coliform bacteria and E. coli. These data agree with the reduction of bacteria expected for the activated sludge system, ranging from 1 to 2 log inactivation (Wen et al. 2009).

Among the disinfection processes evaluated, peroxidation was the least efficient at bacteria inactivation. Other authors found no inactivation of E. coli exposed to hydrogen peroxide concentrations between 25 and 40 mg L\(^{-1}\) with contact times of 60 min (Mamane et al. 2007; Labas et al. 2008). The UV process resulted in a 3 log reduction for both bacteria studied, TC and EC. UV disinfection systems have been used in many wastewater treatment plants in North America and Europe. UV applications are based on several studies which report a minimum UV dose of 1.2 mJ cm\(^{-2}\) to reach a 6 log reduction of E. coli and more than 30 mJ cm\(^{-2}\) to prevent photoreactivation (Guo et al. 2009). Bacteria inactivation data shows that H\(_2\)O\(_2\) assisted by UV is very efficient at inactivation, reaching 4 log reduction for both bacteria. These results show an increase of 1 log in inactivating the bacteria when H\(_2\)O\(_2\) and UV are used together.

An average of 500 helminth eggs and 1,157 larvae were found per liter of influent samples, while 80 eggs and 197 larvae were found in each liter of effluent. The helminth eggs observed were of the genera Ascaris and hookworm. However, all eggs were fertilized (viable) when analyzed by Trypan Blue staining. Embryonated eggs of hookworms were seen.

The activated sludge system also had an average reduction of helminth eggs and larvae of less than 1 log. Wen et al. (2009) reported a reduction of almost 3 log in the eggs of Ascaris spp. in an activated sludge laboratory system. However, the authors claimed that this high reduction might have been affected by the hydraulic conditions in the laboratory system.

A great variation in the number of organisms can be seen in the samples in this study, both of eggs and larvae. This fact shows the difficulty of working with a real sample for detection and study of helminths in order to quantify the organisms, since there are many variables that have a direct influence, mainly seasonality, the amount of suspended solids and solids that settle, and wastewater treatment plant influent flow. The average helminth eggs and/or larvae observed in samples of effluent (disinfected or not) are shown in Tables 3 and 4. The standard deviation values confirm that there is a high variability in quantification of helminths.

The detention time of raw sewage in the activated sludge process at Samambaia WWTP is about 48 hours. This could explain why a great number of helminth larvae are seen. Helminth eggs at this point can adhere to particulate matter and have been sedimented. This prevents them from being counted accurately since the supernatant was evaluated.

Even considering these variations, the peroxidation process assisted by ultraviolet radiation was more efficient than the UV and peroxidation processes for helminth eggs. Although a larger number of helminth larvae were observed in samples disinfected by H\(_2\)O\(_2\)/UV than by the H\(_2\)O\(_2\) or UV processes, no statistically significant difference was found (\(p = 0.05\)) between the data obtained from the three processes.

### CONCLUSIONS

Using an activated sludge system, the Samambaia WWTP has reached the expected results in terms of reduction of numbers of pathogens, considering the control data from treated effluent. H\(_2\)O\(_2\)/UV was more effective at reducing helminth eggs and inactivating bacteria. Thus, the H\(_2\)O\(_2\)/UV advanced oxidation process has a great potential as a step disinfection technique to control total coliform, Escherichia coli bacteria, helminth eggs, and helminth larvae present in treated sewage and to minimize contamination of the receiving waters in order to protect public health.

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