Acta Scientiarum

http://www.uem.br/acta
ISSN printed: 1806-2563
ISSN on-line: 1807-8664
Doi: 10.4025/actascitechnol.v37i2.24950

Study of sequential disinfection for the inactivation of protozoa and indicator microorganisms in wastewater

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ABSTRACT. Sewage disinfection has the primary objective of inactivating pathogenic organisms to prevent the dissemination of waterborne diseases. This study analyzed individual disinfection, with chlorine and ultraviolet radiation, and sequential disinfection (chlorine-UV radiation). The tests were conducted with anaerobic effluent in batch, in laboratory scale, with two dosages of chlorine (10 and 20 mg L^{-1}) and UV (2.5 and 6.1 Wh m^{-3}). In addition, to guarantee the presence of cysts in the tests, 10^4 cysts per liter of *Giardia* spp. were inoculated. The resistance order was as follows: *E. coli* = Total Coliforms < *Clostridium perfringens* < *Giardia* spp.. Furthermore, synergistic effects reached 0.06 to 1.42 log of inactivation in sequential disinfection for both the most resistant microorganisms.

Keywords: chlorine, ultraviolet radiation, *Giardia*, *Clostridium perfringens*, total coliforms, synergism.

Estudo de desinfecção sequencial para inativação de protozoário e microrganismos indicadores em esgoto sanitário

RESUMO. A desinfecção de esgoto sanitário tem o objetivo principal de inativar microrganismos patogênicos para combater a disseminação de doenças de veiculação hídrica. Este estudo analisou a desinfecção individual, com cloro e radiação ultravioleta, e a desinfecção sequencial (cloro - radiação UV). Os testes foram realizados com o efluente anaeróbio, em escala de bancada e em batelada, com duas doses de cloro (10 e 20 mg L^{-1}) e duas doses de radiação UV (2.5 e 6.1 Wh m^{-3}). Além disso, para os testes, a fim de garantir a presença de cistos, foram inoculados 10^4 cistos por litro de *Giardia* spp. A ordem de resistência foi a seguinte: *E. coli* = coliformes totais < *Clostridium perfringens* < *Giardia* spp. Além disso, na desinfecção sequencial para ambos os microrganismos mais resistentes, os efeitos sinérgicos alcançaram de 0,06 a 1,42 log de inativação.

Palavras-chave: cloro; radiação ultravioleta; *Giardia*; *Clostridium perfringens*; coliformes totais; sinergismo.

Introduction

Giardia lamblia (synonyms: *G. duodenalis* and *G. intestinalis*) is a flagellated protozoan that was discovered by Antonie van Leeuwenhoek in 1681. This microorganism raises concerns due to its low infective dose and survival in unfavorable environments, being released in great numbers by ill and asymptomatic individuals. It is an obligatory parasite and the most associated with diarrheic diseases in the world, presented as a cyst in its resistant form (OKOH et al., 2007; PLUTZER et al., 2010).

Sewage, depending on the type of treatment process, presents high concentration of *Giardia* cysts. In raw sewage, 10^2 to 10^5 cysts per liter are normally found (ROBERTSON et al., 2006; CANTUSIO NETO et al., 2006). After secondary treatment of sewage, such as activated sludge, approximately 5.9 to 79 cysts per liter are usually reported (CASTRO-HERMIDA et al., 2008; MOULIN et al., 2010).

Persistence in the aquatic environment, resistance to disinfection by chlorine and infectivity are characteristics that make *Giardia* spp. a high-risk pathogen for public health (BETANCOURT; ROSE, 2004). Therefore, an efficient sewage treatment process with high removal rate and microorganism inactivation decreases the amount of pathogens discharged in water sources (CASTRO-HERMIDA et al., 2008; LEVANTESI et al., 2010).

Chlorine is the most widely used disinfectant in the world for treating water and wastewater. With the discovery of chlorine byproducts, ultraviolet radiation became more popular (BV, 2010). The disinfection mechanism through UV light is based on changes in the DNA and the RNA of microorganisms. UV radiation has the advantage of

not leaving any residual in the treated sewage, and its effectiveness is not affected by pH or temperature (HIJNEN et al., 2006; BV, 2010).

In sequential disinfection, the effect of the combined action of disinfectants, which promotes an inactivation of microorganisms that is higher than the sum of the inactivation of the same disinfectants applied separately, is called synergism (USEPA, 1999; CHO et al., 2010). The probable reasons for the increased interest in these processes are the use of smaller concentrations of disinfectants and the possible decrease in the formation of disinfection byproducts, the control of the formation of biofilms in the water distribution system and the high inactivation levels of pathogenic microorganisms, including the most resistant types, such as *Giardia* spp. and *Cryptosporidium* spp. (USEPA, 1999).

Thus, the goal of this study was to evaluate and compare the resistance of *Giardia* spp. and indicator microorganisms - total coliforms, *Escherichia coli* and *Clostridium perfringens* - to disinfection with chlorine, ultraviolet radiation, and chlorine followed by ultraviolet radiation, with the evaluation of synergistic effects.

Material and methods

The sewage used in the study experiments was obtained from the São Paulo University Wastewater Treatment Plant (WWTP) in São Carlos, which has preliminary treatment composed of screening, sand and a grease removal tank and an Upflow Anaerobic Sludge Blanket (UASB) reactor with 18.8 m³ of operating volume. Table 1 presents the characteristics of the treated wastewater used in the study.

Table 1. Anaerobically treated wastewater used in the disinfection tests.

Variable	Mean ± standard deviation
Temperature	22.5 (± 1.5)
pH	6.85 (± 0.24)
COD (mg L^{-1})	506 (± 98)
N-NH_3 (mg L^{-1})	52.6 (± 13)
Abs 254 nm	0.840 (± 0.12)
TS (mg L^{-1})	532 (± 90)
TSS (mg L^{-1})	139 (± 49)
TC (CFU mL^{-1})*	1.0×10^5
E. coli (CFU mL^{-1})*	1.7×10^4
CP (CFU 100 mL^{-1})*	2.7×10^4

Notes: CFU: colony forming unit. *Geometric mean; TS: total solids; TSS: total suspended solids; TC: total coliforms; CP: *Clostridium perfringens*.

The disinfection tests were conducted in bench units, in batches, with inoculation of approximately 10^4 *Giardia* spp. cysts per liter. Aliquots of 600 mL were retrieved as samples for the analysis.

Disinfection with chlorine

Borosilicate glass beakers with capacity of two L were disinfected and washed with a Tween 80 (0.1%) solution. After the sewage was added, the containers were placed in a jar test apparatus and submitted to a speed gradient of 100 s^{-1}. A solution of Sodium hypochlorite (NaOCl – analytical grade) was prepared on the day of the tests from a stock solution of 4 to 6% P.A. (Vetec Química Fina, Ltda). The final free chlorine concentration used was analyzed by the DPD colorimetric method. The dosages used were: 10 mg L^{-1} for 10 min. of contact time; 10 mg L^{-1} for 20 min. and 20 mg L^{-1} for 10 min.

Disinfection with ultraviolet radiation

Prior to the tests, the average intensity of radiation emitted by the lamps inside the reactor was determined through radiometry (UVX Radiometer – sensor for 254 nm – UVP, CA, USA) in nine evenly distributed points, with the reactor empty. The correction of average intensity on the liquid was carried out considering wastewater radiation absorbance (average in a DR 4000 spectrophotometer) and depth of 3 cm; the applied dosage (Wh m^{-3}) was estimated using the corrected average intensity that had been previously calculated. A volume of 5.4 L was prepared in a UV radiation reactor that had been previously disinfected and washed with a Tween 80 (0.1%) solution, and six 15W low pressure mercury lamps that were emerged from the liquid. After the absorption reading reached 254 nm (DR 4000 – Hach), the times to obtain received doses of 2.5 and 6.1 Wh m^{-3} were established.

Sequential disinfection

After testing with chlorine, an intermediary sample was removed (chlorinated sewage). The remaining sewage was sent to the UV radiation reactor. Figure 1 shows the disinfection test scheme.

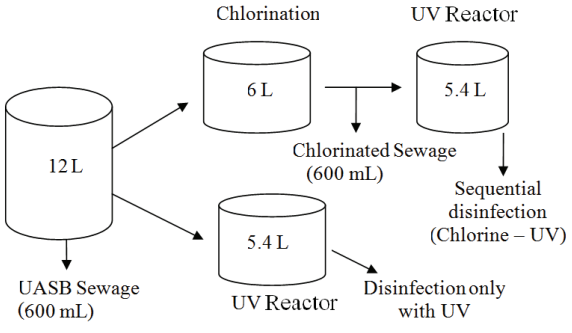

Figure 1. Scheme of the disinfection tests.

Determination of total and free residual Chlorine

The total and free residual chlorine analyses were conducted by the DPD colorimetric method (*N, N*-diethyl-*p*-phenylenediamine) using the spectrophotometer DR 2010 (Hach). The reagents used were DPD Total Chlorine Reagent– CAT 14064-99 and DPD Reactive for free Chlorine – CAT 14070-99 (Permachem®Reagentes, Hach). The DPD was oxidized by the chlorine present in the sample with the formation of a pink solution, which had an intensity that was proportional to chlorine concentration.

At the end of each test, a 3% sodium metabisulfite solution ($Na_2S_2O_5$) was used in a proportion of 0.1 mL for each 100 mL of the sample to remove the combined and free residual chlorine.

Microbiological exams

Giardia spp.

The methodology was adapted from McCuin and Clancy (2005) and Robertson et al. (2000). For concentration of cysts, two centrifugation steps were used at 1500 g for 15 min. from a 75 mL sewage sample with 25 mL Tween 80 (0.1%). Supernatant was removed keeping a volume of 5 mL and no more than 500 µL of sediment. At the stage of purification by immunomagnetic separation (IMS), the Invitrogen Dynal AS kit (Norway) was used following the manufacturer's protocol with the use of dynabeads specific for *Giardia* (Dynal CG – Combo anti-*Cryptosporidium* and *Giardia*). The acid dissociation of cysts was performed with HCl (0.1 mol L^{-1}). The detection with immunofluorescence assay (IFA) performed with the Merifluor kit (Meridien Bioscience, Cincinnati, Ohio) and DAPI solution (4',6-diamidino-2-phenyl-indol) (DAPI - Sigma®), with a 1:4000 dilution.

The viability was tested through vital dye assay with propidium iodide (Sigma-Aldrich, USA). The reagent responsible for the emission of red fluorescence (λ = 510 to 550 nm) penetrates only the microorganisms with a damaged membrane (dead cells). The recovery of cysts reached a value of 53.1 (± 29.7%), which was within the values predicted in the 1623.1 method (USEPA, 2012). The samples were examined with an immunofluorescence microscope (Olympus BX51) with an increase in magnification from 400 to 800 X. The result was expressed as the number of cysts per liter.

E. coli and total coliforms

For the quantification of these microorganisms, the pour plate technique was used with Chromocult® Coliform Agar medium (Merk Cat.No.1.10426). According to Olstadt et al. (2007), Chromocult® Coliform Agar medium has the ability to detect the presence/absence of total coliforms and *E. coli* and to enumerate organisms. The Petri dishes were subsequently incubated at 36 ± 1ºC for 24 ± 1 hours. This methodology was used due to the amount of solids in the membrane that make the identification of colonies difficult. The colonies that show a dark purplish blue color were identified as *E. coli*, whereas those that revealed a reddish salmon color added to the number of *E. coli* colonies, were identified as total coliforms. The result was given in CFU by mL.

A comparison of this technique with the membrane filter technique (APHA et al., 2005) was performed. The statistical results from the ANOVA test indicated that there was no significant difference between the two methods for *E. coli* and total coliforms counts (P-value = 0.87 and 0.14 for total coliforms and *E. coli*, respectively) (Statistica 7.0, StatSoft, Inc).

Clostridium perfringens

For the detection of this pathogen, the membrane filter technique was performed next, with a mCP agar. It is also the method used by Massé et al. (2011). Muller-Spitz et al. (2010) demonstrated that the mCP method is a robust approach to enumerate and isolate *C. perfringens* from samples receiving faecal pollution. First, the sample in decimal dilutions was heated in a water bath at 60 ± 0.5ºC for 15 min. to eliminate any non-spore-forming organisms and vegetative forms. Subsequently, the sample was immediately cooled in an ice bath.

Subsequently, filtering of 100 mL of the sample was performed through a sterile cellulose nitrate membrane with 0.45 µm porosity (Sartorius Stedim Biotech GmbH, Germany), and the sample was then transferred to a disposable sterile Petri dish containing a selective and differential culture medium m-CP agar (Oxoid Ltda, England). Subsequently, the dishes were incubated in an anaerobiosis jar (Permution, Brazil; Gas Pak® System, BBL) for 24 hours at 44.5±0.2ºC. Maintenance of an anaerobic atmosphere was achieved by inserting an anaerobic system envelope (BD BBLTM GasPakTM Plus, ref. 271040; Becton, Dickinson and Company, USA).

The checking of the colonies was made in a culture medium containing a Thioglycolate Broth (CM 0173, Oxoid Ltd, England), which was incubated at 35±0.5ºC for 24 hours. From the positive tubes with thioglycolate broth, 1 mL was

inoculated in tubes containing a milk medium with modified iron, and the tubes were incubated at 44.5 ± 0.2ºC for 2 hours. The reading of the confirmatory test was conducted with positive tubes, which showed the production of turbulent fermentation in a medium with milk and modified iron, with formation of clot. The result was expressed in CFU per 100 mL.

Result and discussion

Table 2 presents the results of the tests conducted in this study. It is worth emphasizing that to quantify the inactivation in the cases where the number of organisms was below the detection limits (100% of inactivation), the occurrence of one colony forming unit (CFU) was considered for the calculations.

Table 2. Inactivation of microorganisms with chlorine, UV radiation and sequential (chlorine-UV radiation).

	Treatments	Total Colif.	*E. coli*	C P	*Giardia*
		Inactivation (-log N No^{-1})			
I	Chlorination (10 mg L^{-1}; 10 min.)	4.30	4.3	0.54	0.09
	UV (2.5 Wh m^{-3})	4.67	4.3	1.84	0.02
	Sequential Disinfection	5.15	4.3	1.94	0.23
II	Chlorination (10 mg L^{-1}; 10 min.)	3.7	4.6	0.02	
	UV (2.5 Wh m^{-3})	3.0	3.6	2.32	
	Sequential Disinfection[1]	5.7	4.6	1.48	
	UV (6.1 Wh m^{-3})	4.7	4.6	2.02	
	Sequential Disinfection[2]	5.7	4.6	1.48	
III	Chlorination (10 mg L^{-1}; 20 min.)	4.95	4.43	0.24	
	UV (2.5 Wh m^{-3})	3.17	3.73	1.49	
	Sequential Disinfection[1]	4.95	4.43	1.32	
IV	Chlorination (10 mg L^{-1}; 20 min.)	2.90	4.36	1.02	0.00
	UV (2.5 Wh m^{-3})	3.50	4.36	1.24	0.00
	Sequential Disinfection[1]	3.60	4.36	2.32	0.09
	UV (6.1 Wh m^{-3})	4.52	4.36	2.02	
	Sequential Disinfection[2]	4.80	4.36	2.32	
V	Chlorination (20 mg L^{-1}; 10 min.)	4.95	4.43	0.70	
	UV (2.5 Wh m^{-3})	3.17	3.73	1.49	
	Sequential Disinfection[1]	4.95	4.43	2.74	
VI	Chlorination (20 mg L^{-1}; 10 min.)	4.40	3.48	1.18	0.16
	UV (2.5 Wh m^{-3})	3.06	3.18	1.73	0.15
	Sequential Disinfection[1]	4.40	3.48	3.43	0.18
	UV (6.1 Wh m^{-3})	4.40	3.48	1.83	
	Sequential Disinfection[2]	4.40	3.48	4.43	

[1]Sequential Disinfection with chlorine followed by UV dosage of 2.5 Wh m^{-3}. [2]Sequential Disinfection with chlorine followed by UV dosage of 6.1 Wh m^{-3}.

Disinfection with chlorine, considering the concentrations used and repetitions applied, obtained 100% effectiveness in inactivating *E. coli* in all the tests and total coliforms in tests III, V and VI. The spore forming bacteria - *Clostridium perfringens* - proved to be very resistant to chlorine, as also shown by Tyrrel et al. (1995), because only when CT (concentration x contact time) values were applied to 200 mg min^{-1} L^{-1}, above 1 log of inactivation was obtained (tests IV and VI). Nevertheless, in test V, where the same CT value was used, the same effectiveness was not obtained. This may have occurred due to the characteristics of the sewage.

Concerning *Giardia* spp., chlorine was not very effective, with a maximum of 0.16 log of inactivation (test VI). Rice et al. (1982) found, in a study conducted by *in vitro* excystation, great resistance of *Giardia* ssp. cysts, where CT of 75 mg min^{-1} L^{-1} was needed for a 2 log inactivation in pH 7.0 and at 5ºC. Li et al. (2004) found CT values similar to those used in this study, between 0.9 and 1.2 log of *Giardia* spp. inactivation using free chlorine. They also reported that chlorine first acts at the cyst wall resulting in an increase in cyst permeability followed by plasma membrane disintegration and cytoplasm damage, which is highly valuable for ultraviolet radiation sequential application.

In the tests that applied two doses of UV radiation, the same resistance standard was observed with *E. coli* and the coliforms presenting the higher inactivation. The use of a second dose of UV radiation provided a complete inactivation of *E. coli* in the three tests (II, IV and VI), and complete inactivation of total coliforms in the sixth test, leading to an inactivation increase of up to 1.7 logarithmic units when compared to the first dose. *Clostridium perfringens* again proved to be resistant to a second dose of UV radiation, implying in the inactivation of a maximum of 0.78 log more than the first dose in the fourth test. For *Giardia* it reached a maximum of 0.15 log of inactivation.

Liberti et al. (2002) used doses from 100 to 160 mWs cm^{-2} to obtain approximately 60% of *Giardia* ssp. removal. Cantusio Neto et al. (2006) found infective cysts after doses of 25 to 30 mJ cm^{-2} in 33% of the animals submitted to *in vivo* assays. Li et al. (2009) performing *in vivo* infectivity could not obtain *Giardia* ssp. cysts infectivity to decrease more than 1 log after the application of ultraviolet radiation with doses from 18 to 57 mJ cm^{-2}.

As for the tests with sequential disinfection with chlorine and UV (2.5 Wh m^{-3}), *E. coli*, which was inactivated below the detection limit with the application of chlorine only, maintained the same inactivation (100%) in the sequential tests. Total coliforms were 100% inactivated, except for test IV, where the inactivation was of 3.6 log. *Clostridium perfringens* showed an increase in inactivation in the sequential tests reaching up to 3.43 log in test V. For the *Giardia* spp., up to 0.23 log of inactivation was reached in test I.

The second doses of UV in the chlorine sequence provided a more complete total coliform inactivation. Tests II and IV maintained the same inactivation of *Clostridium perfringens* promoted by the first UV dose; however, in test VI, this bacteria

was 100 % inactivated above 4.43 log. Despite presenting the same CT at 200 mg $min.^{-1}$ L^{-1}, test VI resulted in a higher inactivation rate than test IV, and in this case, the dose seemed to be more important than time contact.

Sequential disinfection studies are still incipient for wastewater effluents; however, many articles for sequential disinfection of water may be found in literature. Synergism only occurred for more resistant microorganisms– *Clostridium perfringens* and *Giardia* spp, as shown in Table 3, since the *E. coli* bacteria and total coliforms were often completely inactivated with the application of only one of the used disinfectants.

Table 3. Synergism in sequential disinfection Chlorine – UV Radiation.

Microorganism	Test	Sequential disinfection	∑ Individual inactivation (Ii)	Sequential Inactivation (Si)	*Synergism
C. perfringens	V	Cl – UV1	2.19	2.74	0.55
C. perfringens	IV	Cl – UV1	2.26	2.32	0.06
C. perfringens	VI	Cl – UV1	2.91	3.43	0.52
C. perfringens	VI	Cl – UV2	3.01	4.43	1.42
Giardia spp.	I	Cl – UV1	0.11	0.23	0.12
Giardia spp.	IV	Cl – UV1	0	0.09	0.09

Notes: UV1: 2.5 Wh m^{-3}; UV2: 6.1 Wh m^{-3}; Inactivation Values and synergism are given as log values. *Synergism = Observed Si - ($\sum Ii$) (USEPA; 1999).

Caretti and Lubello (2003) found synergic effects using peracetic acid followed by UV radiation in the disinfection of fecal and total coliforms, *E. coli*, fecal streptococcus and *Pseudomonas aeruginosa* in wastewater. Ryu et al. (2007), after analyzing samples from tertiary effluents of seven WWTPs, concluded that WWTPs with combined disinfection of chlorine followed by UV radiation reached the target for annual acceptable risk for *Giardia* ssp. infection – one infection for each ten thousand inhabitants. Souza and Daniel (2011) found synergistic effects when low doses of chlorine were applied combined with ozone for *E. coli* disinfection.

Wang et al. (2012) reported the occurrence of synergistic effects for heterotrophic bacteria count, total bacterial count and total coliforms in sequential disinfection composed of ultraviolet radiation followed by chlorination of effluent for reuse. According to Koivunen and Heinonen-Tanski (2005), the synergic action can be explained by the mechanism of multiple damage, two different disinfectants can cause damage to different types of microorganisms and, therefore, promote a more effective inactivation of them.

The lower level of inactivation shown by *Giardia* spp. may be explained by an underestimation of the vital dye method, as reported by Campbell and Wallis (2002), in comparison to *in vivo* infectivity. These authors, along with Shin et al. (2009), noticed a greater effectiveness of UV radiation in the inactivation of this protozoan – above 2 log of inactivation – for doses close to the ones used in this study.

However, in the study of Li et al. (2009), even with *in vivo* assays, the decrease in the infectivity of *Giardia* ssp. cysts was lower than 1 log. The same authors report that these results may be due to the cysts protection from UV radiation provided by particles present in the wastewater, cysts reactivation potential after exposure to UV light and bacteria attack and adsorption of colloidal material from cyst surface, partially not permitting UV radiation to penetrate the cysts.

Heaselgrave and Kilvington (2011) reported that although the vital dye PI underestimate inactivation especially for disinfection assays, this problem highlights the requirement for robust and reproducible *in vitro* tests for determination of viability. Current cell culture-based methods are technically difficult to perform and the gold standard for determining the viability of cysts requires the use of *in vivo* animal infectivity studies which are complex, expensive, time demanding and involve ethical questions (SCHETS et al., 2005).

Conclusion

It is possible to use smaller CT for primary disinfection with chlorine, since sequential disinfection may be present inactivation similar to the one obtained with the use of only one disinfectant at higher CT.

Giardia spp. proved to be insusceptible even to sequential disinfection and synergistic effect was noticeable for *C. perfringens* and *Giardia* spp. in some of the tests.

E. coli and total coliforms were not particularly resistant to the disinfectants used, implicating in the questioning of the actual range reached by the traditional indicators.

Acknowledgements

To Fapesp (processes 2007/08499-3 and 2010/07902-1), to CNPq, and to the Laboratory of Advanced Treatment and Water Reuse (Latar).

References

APHA/AWWA/WEF-American Public Health Association/ American Water Works Association/Water Environment Federation. **Standard methods for the examination of water and wastewater**. 21st ed. Washington, D.C.: APHA, 2005.

BETANCOURT, W. Q.; ROSE, J. B. Drinking water treatment process for removal of *Cryptosporidium* and *Giardia*. **Veterinary Parasitology**, v. 126, p. 219-234, 2004.

BV-Black and Veatch Corporation. **Revised edition of: Handbook of chlorination and alternative disinfectants** - 4th ed. 1999. 5th ed. New Jersey: BV, 2010.

CAMPBELL, A. T.; WALLIS, P. The effect of UV irradiation on human-derived *Giardia lamblia* cysts. **Water Research**, v. 36, p. 963-969, 2002.

CANTUSIO NETO, R.; SANTOS, L. U.; FRANCO, R. M. B. Evaluation of activated sludge treatment and the efficiency of the disinfection of *Giardia* species cysts and *Cryptosporidium* oocysts by UV at a sludge treatment plant in Campinas, south-east Brazil. **Water Science and Technology**, v. 53, n. 3, p. 89-94, 2006.

CARETTI, C.; LUBELLO, C. Wastewater disinfection with PAA and UV combined treatment: a pilot plant study. **Water Research**, v. 37, p. 2365-2371, 2003.

CASTRO-HERMIDA, J. A.; PRESSEDO, I. G.; ALMEIDA, A.; WARLETA, M. G.; COSTA, J. M. C.; MEZO, M. Contribution of treated wastewater to the contamination of recreational river areas with *Cryptosporidium* spp. and *Giardia duodenalis*. **Water Research**, v. 42, p. 3528-3538, 2008.

CHO, M.; KIM, J.; KIM, J. Y.; YOON, J.; KIM, J. Mechanisms of *Escherichia coli* inactivation by several disinfectants. **Water Research**, v. 44, p. 3410-3418, 2010.

HEASELGRAVE, W.; KILVINGTON, S. The efficacy of simulated solar disinfection (SODIS) against *Ascaris*, *Giardia*, *Acanthamoeba*, *Naegleria*, *Entamoeba* and *Cryptosporidium*. **Acta Tropica**, v. 119, n. 2-3, p. 138-143, 2011.

HIJNEN, W. A. M.; BEERENDONK, E. F.; MEDEMA, G. J. Inactivation credit of UV radiation for viruses, bacteria and protozoan (oo)cysts in water: A review. **Water Research**, v. 40, p. 3-22, 2006.

KOIVUNEN, J.; HEINONEN-TANSKI, H. Inactivation of enteric microorganisms with chemical disinfectants, UV irradiation and combined chemical/UV treatments. **Water Research**, v. 39, p. 1519-1526, 2005.

LEVANTESI, C.; LA MANTIA, R.; MASCIOPINTO, C.; BOCKLMANN, U.; AYUSO-GABELLA, M. N.; SALGOT, M.; TANDOI, V.; VAN HOUTTE, E.; WINTGENS, T.; GROHMANN, E. Quantification of pathogenic microorganisms and microbial indicators in three wastewater reclamation and managed aquifer recharge facilities in Europe. **Science of the Total Environment**, v. 408, p. 4923-4930, 2010.

LI, Y.; CRAIK, S. A.; SMITH, D. W.; BELOSEVIC, M. Infectivity of *Giardia lamblia* cysts obtained from wastewater treated with ultraviolet light. **Water Research**, v. 43, p. 3037-3046, 2009.

LI, Y.; SMITH, D. W.; BELOSEVIC, M. Morphological changes of *Giardia lamblia* cysts after treatment with ozone and chlorine. **Journal of Envionmental Engineering and Science**, v. 3, p. 495-506, 2004.

LIBERTI, L.; NOTARNICOLA, M.; PETRUZZELLI, D. Advanced treatment for municipal wastewater reuse in agriculture: UV disinfection: parasite removal and by-product formation. **Desalination**, v. 152, p. 315-324, 2002.

MASSÉ, D.; GILBERT, Y.; TOPP, E. Pathogen removal in farm-scale psychrophilic anaerobic digesters processing swine manure. **Bioresource Technology**, v. 102, p. 641-646, 2011.

McCUIN, R. M.; CLANCY, J. L. Methods for the recovery, isolation and detection of *Cryptosporidium* oocysts in wastewater. **Journal of Microbiological Methods**, v. 63, p. 73-88, 2005.

MOULIN, L.; RICAHRD, F.; STEFANIA, S.; GOULET, M.; GOSSELIN, S.; GONÇALVES, A.; ROCHER, V.; PAFFONI, C.; DUMÈTRE, A. Contribution of treated wastewater to the microbiological quality of Seine River in Paris**. Water Research**, v. 44, p. 5222-5231, 2010.

MULLER-SPITZ, S. R.; STEWART, L. B.; MCLELLAN, S. L. Reliability of mCP method for identification of *Clostridium perfringens* from faecal polluted aquatic environments. **Journal of Applied Microbiology**, v. 108, p. 1994-2002, 2010.

OKOH, A. I.; ODJADJARE, E. E.; IGBINOSA, E. O.; OSODE, A. N. Wastewater treatment plants as a source of microbial pathogens in receiving watersheds. **African Journal of Biotechnology**, v. 6, n. 25, p. 2932-2944, 2007.

OLSTADT, J.; SCHAUER, J. J.; STANDRIDGE, J.; KLUENDER, S. A comparison of ten USEPA approved total coliform/*E.coli* tests. **Journal of Water and Health**, v. 5, n. 2, p. 267-282, 2007.

PLUTZER, J.; ONGERTH, J.; KARANIS, P. *Giardia* toxonomy, phylogeny and epidemiology: Facts and open questions. **International Journal of Hygiene and Environmental Health**, v. 213, p. 321-333, 2010.

RICE, E. W.; HOFF, J. C.; SHAEFFER III, F. W. Inactivation of *Giardia* cysts by chlorine. **Applied and Environmental Microbiology**, v. 43, n. 1, p. 250-251, 1982.

ROBERTSON, L. J.; HERMANSEN, L.; GJERDE, B. K. Occurance of *Cryptosporidium* oocysts and *Giardia* cysts in Sewage in Norway. **Applied and Environmental Microbiology**, v. 72, n. 8, p. 5297-5303, 2006.

ROBERTSON, L. J.; PATON, C. A.; CAMPBELL, A. T.; SMITH, P. G.; JACKSON, M. H.; GILMOUR, R. A.; BLACK, S. E.; STEVENSON, D. A.; SMITH, H. V. *Giardia* cysts and *Cryptosporidium* oocysts at sewage treatment works in Scotland, UK. **Water Research**, v. 34, n. 8, p. 2310-2322, 2000.

RYU, H.; MENA, K. D.; ABBASZADEGAN, M. Assessment of the risk of infection by *Cryptosporidium* and *Giardia* in non-potable reclaimed water. **Water Science and Technology**, v. 55, n. 1-2, p. 283-290, 2007.

SCHETS, F. M.; ENGELS, G. B.; DURING, M.; RODA-HUSMAN, A. M. Detection of infectious *Cryptosporidium* oocysts by cell culture immunofluorescence assay: Applicability to environmental samples. **Applied and Environmental Microbiology**, v. 71, n. 11, p. 6793-6798, 2005.

SHIN, G. A.; LINDEN, K. G.; FAUBERT, G. Inactivation of *Giardia lamblia* cysts by polychromatic UV. **Letters in Applied Microbiology**, v. 48, p. 790-792, 2009.

SOUZA, J. B.; DANIEL, L. A. Synergism effects for *Escherichia coli* inactivation applying the combined ozone and chlorine disinfection method. **Environmental Technology**, v. 32, n. 11-12, p. 1401-1408, 2011.

TYRREL, S. A.; RIPPEY, S. R.; WATKINS, W. D. Inactivation of bacterial and viral indicators in secondary sewage effluents, using chorine and ozone. **Water Research**, v. 29, n. 11, p. 2483-2490, 1995.

USEPA-Environmental Protection Agency. **Alternative disinfectants and oxidants guidance manual**. New York: Usepa, 1999.

USEPA-US Environmental Protection Agency. **Method 1623.1**: *Cryptosporidium* and *Giardia* in water by filtration/IMS/FA. Office of Water. EPA 816-R-12-001. Cincinnati: Usepa, 2012.

WANG, X.; HU, X.; WANG, H.; HU, C. Synergistic effect of the sequential use of UV irradiation and chlorine to disinfect reclaimed water. **Water Research**, v. 46, p. 1225-1232, 2012.

Received on September 15, 2014.
Accepted on November 10, 2014.