

Activated Sludge and Ozonation Combined System of Sanitary Effluent Treatment to Bacterial and Protozoa Removal - A Case Study

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Abstract

In order to remediate district effluents, two types of treatments were combined that could be an efficient solution for the great problems involving sanitary effluent, water reuse, organic charge reduction and disinfection processes. Chemical and biological parameters of sanitary effluents before and after activated sludge treatment and subsequent oxidative ozone treatment were monitored. The activated sludge treatment significantly reduced the organic charge, observed by monitoring the chemical oxygen demand (COD) and the total organic carbon (TOC). After the treated effluent from the activated sludge underwent a subsequent treatment with ozone, further decreases in the COD and TOC values were observed. Ozone efficiently eliminated (100%) coliforms and *Giardia spp.* cysts, which normally are not eliminated by the activated sludge treatment.

Keywords: activated sludge, coliform, effluent decontamination, *Giardia spp.*, ozonation, sanitary effluent

1. Introduction

Water of low quality must be considered an alternative source for less restricted applications. The appropriate use of technologies for the development of these sources is of paramount importance today. Enhancement of the efficacy in the use and control of the demand is the basic strategy for the solution of the water shortage in the world (ANA, 2006). The alternative sources for the less restricted uses are industrial process effluents, sewage, especially from domestic source, water drainage yards, agricultural and salty waters. The two most important advantages of water reuse are the reduction of contamination of the aquatic body and the economy of productive processes due to their aggregated value (ANA, 2006).

At the moment, there are many options for effluent treatment using chemical, physical and biological processes. The physical processes involve separation stages, filtration, microfiltration, ultrafiltration and flocculation, which all involve simple phase transfers of the effluent contaminants. However, chemical processes, such as ozonation and advanced photocatalytic oxidative processes are able to degrade contaminant. Biological processes, which use microorganisms that can act through oxidative and fermentative mechanisms also transform or degrade the compounds present in the effluent (CETESB, 2000a).

A combination of physical, chemical and biological processes in effluent treatment has proven to be a good solution, resulting in an improvement in the quality of the final effluent after treatment short times (CETESB, 2000a; Mansilla et al., 2007). In the present study, activated sludge was used as a biological treatment and ozonation as chemical one.

According to Santos et al. (2004), a chemical system for effluent treatment using chlorine is currently the most used system. However, this treatment is inefficient in the elimination of *Giardia* cysts (Medeiros, 2010). These cysts, which are eliminated together with the excrements of infected host can pass through biological treatment stations and end up in rivers where they can pollute the drinking water sources and cause severe gastrointestinal disease in humans and animals (Bonatti et al., 2007)

Among other methods, ozone is also used to eliminate *Giardia* such as *Cryptosporidium* (Betancourt & Rose, 2004; Haas & Kaymak, 2003). For this reason, in this work the ozonation of the sanitary effluent previously treated with activated sludge is proposed in order to achieve total disinfection.

The technology in which the activated sludge is used consists basically in the agitation of the effluent in the presence of aerobic bacteria and micrometazoa and atmospheric oxygen for a period necessary to metabolize and to flocculate a large part of the organic material (CETESB, 2000a; von Sperling, 1995, 1997; Reali, 1999).

The most common organisms that participate in the biodegradation of organic material in the biological system of sewer treatment are protozoa, bacteria and annelids (CETESB, 2000a; Cordi et al., 2007; Assalin et al., 2007a, 2007b).

According to the Brazilian Environmental Protection Agency of the State of São Paulo (CETESB, 1990), activated sludge is the most advisable technique for domestic effluent treatment; moreover, the treated effluent can be discharged into the receiving body (rivers or streams) within standards that meet the Environment State Law (São Paulo, 1976, 2005).

To apply the activated sludge process to industrial effluents, which have specific compositions, an effluent characterization is needed before its treatment and the sludge needs to be acclimatized by, for example microorganism adaptation to the effluent to be treated (Reginato et al., 2009). Effluent characterization requires determination of pH, heavy metals, toxic compounds, biochemical oxygen demand (BOD), chemical oxygen demand (COD) and total organic carbon (TOC) (CETESB, 2000; Cordi et al., 2008).

Giardia belongs to Diplomonadida and Hexamitidae families and only *G. duodenalis* is found in humans and in domestic and wild mammals (Thompson, 2004). *Giardia spp.* transmission is via fecal-oral. The Brazilian environmental law does not oblige the environmental treating companies to eliminate cysts and only recommends research on pathogenic organisms. *Giardia spp.*, for example, needs to be completely absent in treated water in USA; that has different rules related to *Giardia* presence in effluents (EPA, 1989).

Ozone has numerous applications in the treatment of effluent and industrial residues (Masten & Davies, 1994; Rice, 1999; Kunz et al., 1999, 2002; Freire et al., 2000, 2001; Bassani, 2003; Vogelpohl & Kim, 2004; Assalin et al., 2004a, 2004b, 2006, 2007, 2009; Almeida et al., 2004; Melo et al., 2006; Moraes et al., 2006; Catalkaya & Kargi, 2007; Assalin & Durán, 2007a; Lima, 2007; Medeiros, 2010; Domenjoud et al., 2011; Rivas et al., 2011). According Mahmoud and Freire (2007), the use of ozone as an oxidation agent has increased in the last decades.

Due to the high efficiency for pathogenic microorganism removal and organic compound oxidation, many countries apply this technique as a disinfectant during water treatment for human consumption (Gottschalk et al., 2000; Hsu & Yeh, 2003; Rojas-Valencia et al., 2004; Santos et al., 2004; Erickson & Ortega, 2006).

Cardoso et al. (2003) used ozone to totally eliminate coliforms and fecal presence (*Escherichia coli*) after 2 min with a 4 mg/L concentration. However, Melcaf and Eddy (2003), using 10-40 mg/L of ozone, Gonçalves (2003) using 2.5 min with a 12 mg/L of ozone, and Lage-Filho (2008), using 12 min with a 2.25 mg/L of ozone, observed reminiscent fractions of coliforms. On the other hands ozone was effective for *Giardia lamblia* cysts in water after 5 min treatment with an ozone concentration of 0.5 mg/L. The first effect was membrane destruction (Finch, 1996).

The aim of this work was to evaluate the effect of combined technologies in the effluent treatment and disinfection by activated sludge and ozonation (followed by COD and TOC), in order to obtain reusable water with a total elimination of *Giardia spp.* cysts in Brazilian water treatment stations.

2. Experimental

2.1 Sample Collections

Ten water samples (classified as A-J) were collected (Table 1). The samples were collected in sterilized glass 2 L bottles from the Samambaia Sewage Treatment Plant, situated in a suburb of the eastern region of Campinas, SP, Brazil, and serving a population of 40,000 (Bonatti, 2007). The samples were collected (December 2006 to February 2007). For each collection, 2 L were taken after the inflow filters and before the activated sludge treatment and 5 L were obtained after activated sludge treatment (outflow) from STS.

Table 1. Sample collected indicating code and date

Sample	Date
A	08/01/2007
B	12/01/2007
C	29/01/2007
D	20/12/2006
E	25/01/2007
F	06/02/2007
H	24/01/2007
I	30/01/2007
J	07/02/2007

2.2 Detection of Coliforms and *Escherichia Coli*

For total coliforms and *Escherichia coli*, the membrane filter method was used (CETESB method – Filtrant membrane method-NT L5.214) (CETESB, 2000b). The results were expressed as colony formation units in 100 mL of sample (CFU/100 mL).

2.3 Detection of *Giardia* Cysts by Membrane Filtration Method

Effluent sample, 1L, were filtered through mixed cellulose ester membranes (45-mm-diameter, 3 µm, Millipore, Brazil) and transferred to Petri dishes. Membranes were scraped with a soft plastic loop for 10 minutes and manually rinsed for 10 minutes with eluting solution (0.1% of Tween 80 solution). The resulting liquid was centrifuged for 15 minutes (650 x g), and the pellet rinsed with filtered water system (MilliQ, Millipore, Brazil) for 15 minutes.

Aliquots (10 µl) of these pellets were processed by Merifluor kits (Meridian Bioscience, Cincinnati, Ohio) according to the manufacturer's instructions (Santos et al., 2004, 2011). A Zeiss Axiolab epifluorescent microscope with a 450-490 nm excitation filter and 520 nm barrier was used to read the reaction. The number of cysts was calculated by [cyst counts in the well x vol. of pellet/sample-well vol.] x 2. (Franco et al., 2001, 2002).

2.4 Chemical Parameters

The effluent chemical composition was characterized using the following parameters: pH, total organic carbon (TOC) and chemical oxygen demand.

A pH meter Orion model EA 940 with a glass combined electrode was used for measuring the pH of effluents during the ozonation process.

The C-content in solution, was determined according to ISO-8245 Total Organic Carbon (TOC-5000A) Shimadzu analyzer (ISO, 1987).

A COD was measured using the procedure of the Standard Methods for the Examination of Water and Wastewater of the American Society of Civil Engineers (APHA 5310 B, 1995).

2.5 Ozonation Procedure

The ozonation was carried out in an ozonator as described by Kunz et al. (1999) (Figure 1). The ozone quantity used was 4 mg/h in 400 mL of effluent for 3 or 5 minutes of treatment.

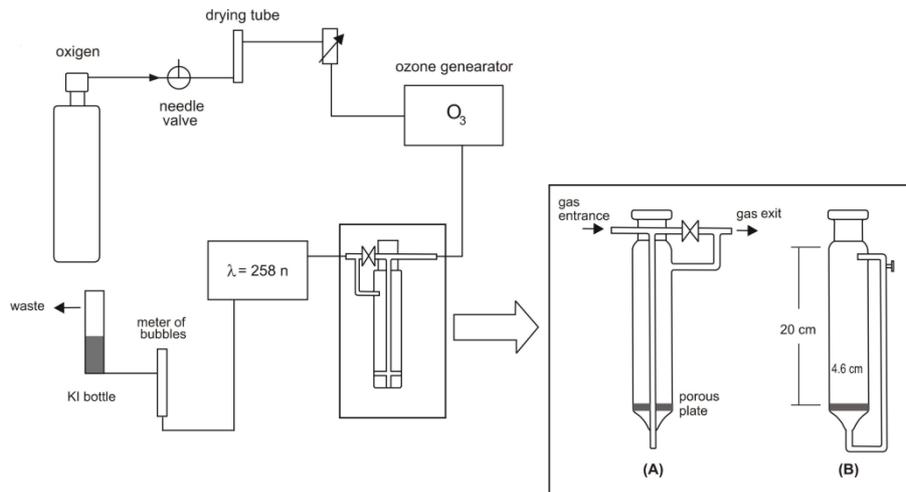


Figure 1. Schematic diagram of the ozonation system (modified from Kunz et al., 1999)

3. Results and Discussion

In the first stage of this work, the chemical characterization of the affluent (before-inflow) and effluent (after-outflow) of the activated sludge treatment was carried out (Figure 3). COD and TOC parameters of each sample were analyzed.

Figure 3 shows the different effluents treatment. The A, B and C samples were ozonized for 3 min; the D, E and F samples were ozonized for 5 min. The H, I and J samples were ozonized for 3 or 5 min, respectively. This procedure was done in order to eliminate the sample heterogeneities and to show that the differences in the results do not depend on effluent variation.

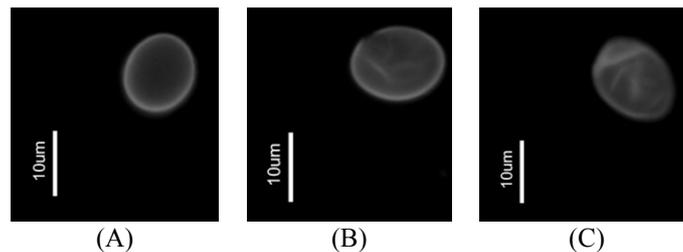


Figure 2. Microphotography of *Giardia spp.* cysts in sample H before ozonation x 400 (A); after 3 min of ozonation (B) and after 5 min of ozonation (C)

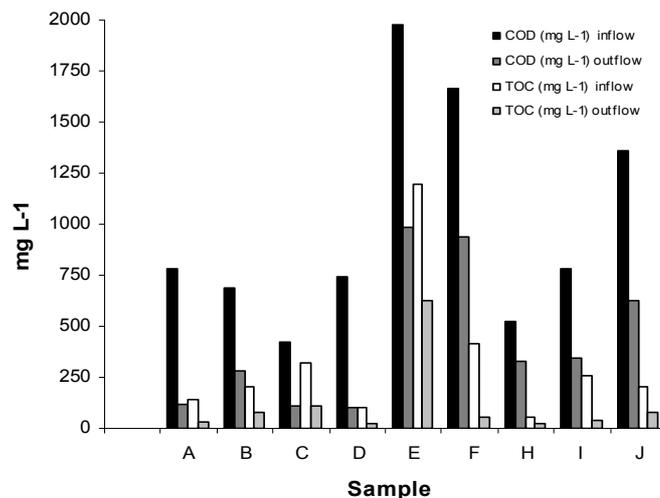


Figure 3. Efficiency of activated sludge treatment followed by COD and TOC ^a

a) In duplicates (deviation error $\pm 10\%$).

A relatively large variation from the different treatment days was observed, due to the large daily variation of the organic materials in the affluent (inflow-sewer). The treatment monitored by COD values exhibited an average value of 63% (minimum value of 28% and a maximum of 85%) of COD removal. Similar variations were observed for TOC values, with an average of 70% (minimum of 48% and a maximum of 86%) of the TOC removal.

The differences of the COD and TOC values are due to the variability of the activated sludge treatment. The COD and TOC parameters in the activated sludge showed values that have reached the expected efficiency as described by Almeida et al. (2004). Activated sludge is an efficient biological method for effluent treatment, but does not consider significant parameter variations, such as organic charge.

The total coliforms and fecal coliforms after activated sludge treatment clearly showed that these microorganisms were not efficiently eliminated in the process. The effluent after this treatment showed 3.8×10^5 CFU of total fecal coliforms/100 mL of effluent. The presence of *Giardia spp.* cysts was monitored and a $[8.2 \pm 4, 2] \times 10^5$ cysts/L of effluent value was found.

After the treatment with activated sludge, ozonation was applied for short periods in order to evaluate the viability of this combined process. The variation of pH with the samples in the absence of ozone was 5.8 to 6.2; after ozonation the pH changed to 6.6 to 6.9. Figure 4 shows the COD values found before and after the ozonation process for 3 min.

A large variability in the COD reduction values was observed between the treated effluent due to the variability of the activated sludge system. This can be ascribed to the different chemical components of the collected samples. The average reduction of COD obtained in the ozone treatment for 3 min was 46 ± 21 %. The values varied from 13% to 89% of COD removal.

Figure 4 show the COD values found before and after ozone treatment for 5 min with an average value of 48% (± 21 %). The values varied from 18% to 87% removal.

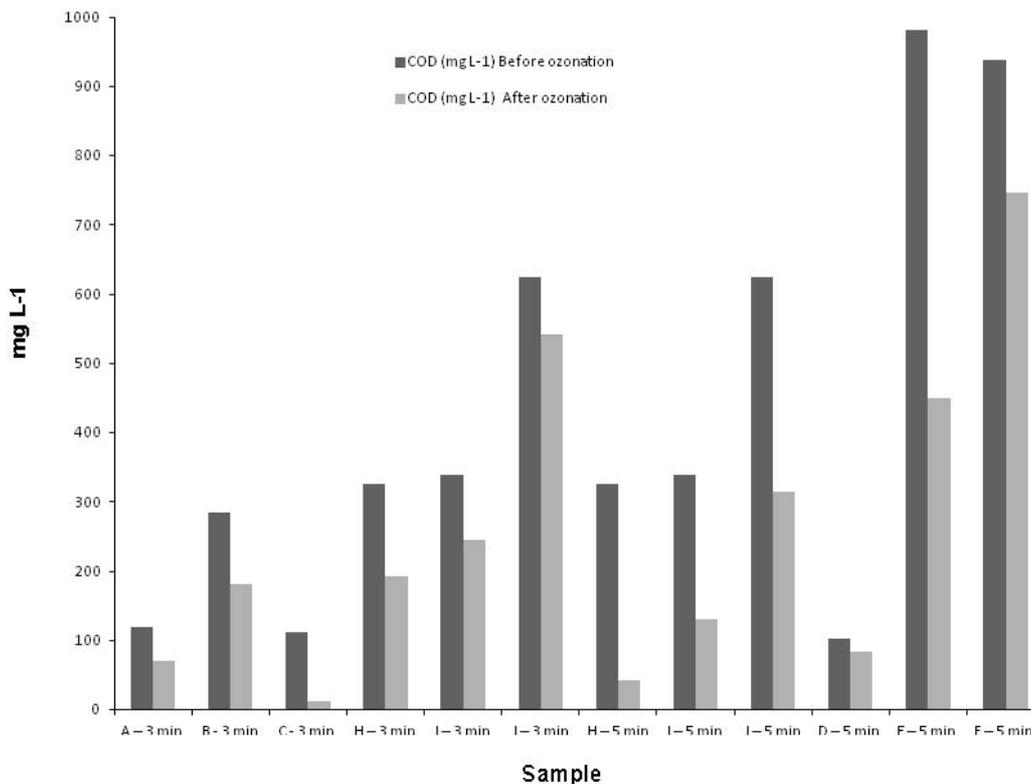


Figure 4. COD values of ozonized samples for 3 min^a and 5 min^b

- a) In duplicates (deviation error ± 10 %). Average value of the samples was 44.4% (± 23.4 %).
- b) In duplicates (deviation error ± 10 %). Average value of the samples was 48% (± 21.2 %).

In general, no large variations were observed in the COD reduction by ozonation after 3 or 5 minutes of treatment. However, if we compare equivalent samples (same collected sample after 3 or 5 min of treatment (Figure 4 and Table 1), it is possible to verify differences. The H, I and J samples, which are the same collected sample, the increases of affectivity were 28.4% (sample H), 33.8% (sample I) and 36.4% (sample H), respectively.

The TOC variations in the ozonation process are similar to those for the COD. Figure 5 shows the TOC values that were found before and after ozonation for 3 and 5 minutes. TOC was very sensitive to the quality of the effluent from the activated sludge but not to the amount of original TOC.

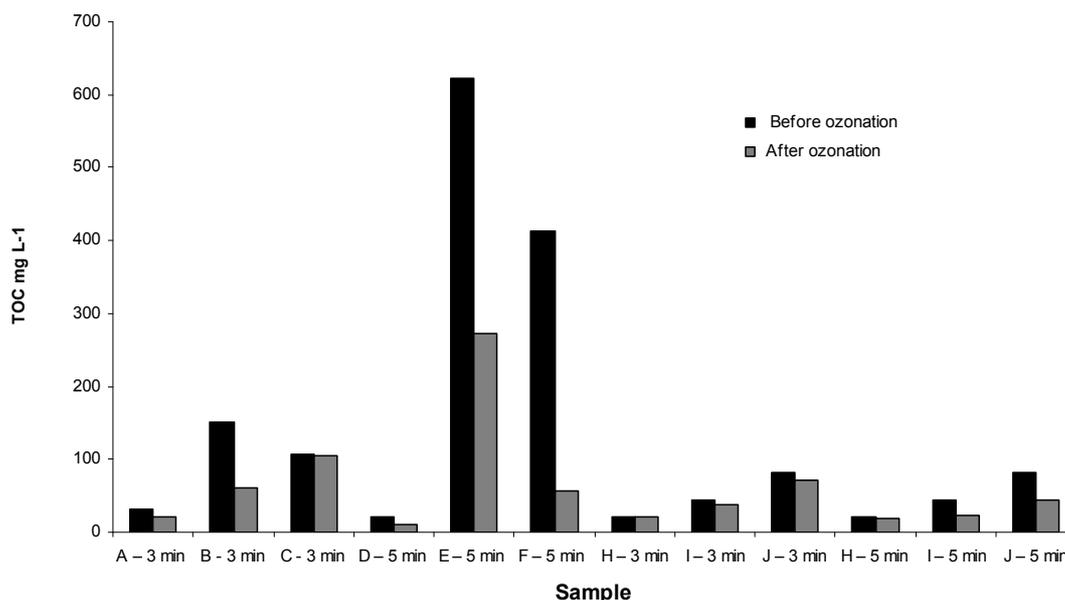


Figure 5. TOC values of ozonized samples for 3 min^a and 5 min^b

- In duplicate (deviation error $\pm 10\%$). Average of the samples was $65 \pm 14\%$.
- In duplicates (deviation error $\pm 8\%$). Average of the samples was $31.2 \pm 19.2\%$.

Figure 5 shows the TOC values found in the effluent before and after ozonation treatment for 3 and 5 min, carried out with the same samples H, I and J, the increase of affectivity were 13.0% (sample H), 30.8% (sample I) and 36.1% (sample H), respectively.

The reduction of COD and TOC values, in general, increased with longer periods of ozonation. After 3 min the minimum values were observed, while for 5 min the reduction values were higher than for 3 min. ozonation, with the best results around 50% reduction.

As stated, the intention was to analyze real samples from different collections without any dilution in order to determine COD or TOC, since the organic components of the affluent and effluents were changing constantly. This was one of the reasons for the large variation in the ozonation process. It was clear that the efficiency does not depend on the COD or TOC values, but on the organic material quality. The low TOC reduction was probably due to incomplete oxidation of the organic compounds. The same type of results was observed with a whey effluent (Almeida, 2004). As expected, ozone was able to clarify the final effluent (Krull et al., 1998; Kunz et al., 2002).

After the analysis of the effluent from the activated sludge treatment before and after ozonation, the degree of disinfection of the affluent and effluent from the activated sludge and from ozonation was studied in order to understand the effectiveness of these two processes in the elimination of bacteria and *Giardia* cysts.

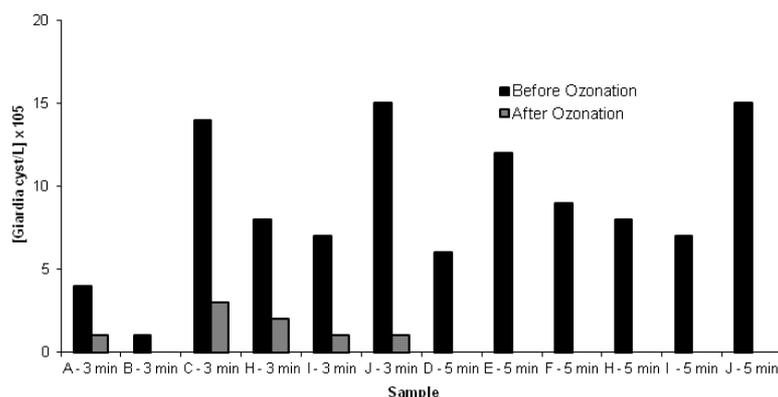
Table 2 shows the CFU in 100 mL of effluent of total coliforms and fecal coliforms, before and after ozonation for 3, 5 and 10 minutes.

Table 2. CFU/100mL of total coliforms and fecal coliforms after ozonation^a

Treatment	Total Coliforms	Fecal Coliforms
Before ozonation	3.8×10^5	3.4×10^5
After 3 min. ozonation	1.7×10^2	0
After 5 min. ozonation	9.5×10^1	0
After 10 min. ozonation	0	0

The results showed that ozone is very efficient against fecal coliform, which was also described by Cardoso et al. (2003), where total fecal coliform elimination after 3 min of ozonation was obtained. At the same time a strong reduction of the total coliforms was observed with total elimination after 10 min of treatment.

Figure 6 shows the *Giardia spp.* cysts found before and after 3 min of effluent ozonation. The 3 min ozonation treatment was not enough for total cyst elimination, with a cyst/L count over 1×10^5 . Figure 6 also shows the *Giardia spp.* cyst analyses after 5 min of ozonation. These results show that the destruction rates of *Giardia spp.* cysts were 100% in six independent assays when 5 min of ozonation was applied.

Figure 6. *Giardia spp.* cysts/L before and after ozonation treatment for 3 min^a and 5 min^a

a) In duplicates (deviation error $\pm 10\%$). The differences of the cyst/L counting are due to variability of the activated sludge treatment.

Finch (1996) showed that ozone caused membrane damage of the *G. lamblia* cyst and when this membrane is broken the cytoplasm leaks out and the cyst dies. However, it is only possible to say that the cyst has died when the ozone completely oxidizes the membrane. In this study, Figure 2A shows the intact membrane and cyst surface before ozonation, in Figure 2B the cyst surface is damaged after 3 min of ozone treatment. In Figure 2C, it is possible to also see membrane damage and reduction of fluorescence after 5 min of ozonation. The damage was evident independent of the sample treatment or collection sample.

Our concern was to follow the global tendency that is being implementing for protozoa treatment, and in this case, *Giardia spp.*, was investigated. In Brazil, the Ministry of Health recommends the monitoring *Giardia*, pathogenic protozoa, in the finished water at Water Treatment Plants. Currently, a major challenge in producing high-quality drinking water is to monitor waterborne pathogens such as *Cryptosporidium spp.* and *Giardia spp.* in water samples (Neto et al., 2010). However, recently a revision of this recommendation was discussed. Chemically assisted direct or conventional filtration and disinfection, and slow sand filtration and disinfection processes operating in accordance with performance criteria, have been credited with a 3-log (99.9%) removal or inactivation of *Giardia* cysts (SDWA, 2006).

4. Conclusions

The activated sludge used in this water treatment station was efficient reducing in the organic material charge of the samples studied under real conditions. However, it was not efficient for protozoa cysts elimination.

An oxidative chemical process using ozone showed potential to reduce the organic charge of the effluent after activated sludge treatment through the COD and TOC parameters and represents a viable alternative for application after biological treatment. Ozonation was a 100% efficient in eliminating bacteria and *Giardia spp.*

cysts with 5 min of ozone treatment. Also these results showed that the combined process was able to reduce the coliforms to the levels required by Brazilian environmental regulations (ANA, 2006).

Based on these results, we hope that Brazil will meet the international standards of water quality, where the protozoa are determined in the assays of effluent and tap waters.

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