Efficacy of Chlorine Dioxide on Inactivation of *C. parvum* Oocysts

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Abstract

Chlorine dioxide (ClO_2) is a broad-spectrum disinfectant that might be an effective alternative to hyperchlorination for inactivation of *Cryptosporidium* in pools. No studies have specifically addressed the effects of ClO_2 on *C. parvum* oocyst infectivity in chlorinated recreational water settings. The aim of this research was to determine the efficacy of 5 mg/L (ppm) ClO_2 and ClO_2 combined with 2 mg/L free chlorine (FC) against *C. parvum* oocysts. CT values of 622 and 489, for 5 mg/L ClO_2 and 5 mg/L ClO_2 with 2 mg/L FC, respectively, were found to be required for a 99.9% reduction of oocysts. Chlorine dioxide at 5 mg/L, both alone and in the presence of 2 mg/L free chlorine, appears to be an effective and efficient alternative to hyperchlorination for inactivating *Cryptosporidium* in recreational water venues, but may be hazardous and must be used safely.

Introduction

Cryptosporidium spp. are coccidian protozoan parasites that infect the cells of the small intestine of humans and other mammals, causing acute or persistent diarrhea. Small, hardy oocysts are excreted in the stool and are ready to initiate infection upon ingestion. Due to the oocysts' resistance to chemical disinfectants (notably chlorine) and propensity for waterborne transmission, *Cryptosporidium* spp. are the leading cause of diarrheal disease outbreaks associated with chlorinated recreational water facilities. Hyperchlorination, or the increase in free chlorine concentration to achieve a CT value of 15,300 mg·min/L, is recommended by CDC following a known or suspected diarrheal incident in these facilities to achieve a 3-log (99.9%) inactivation of *Cryptosporidium* oocysts.

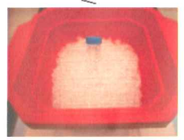
While chlorine is the most universally utilized water disinfectant, chlorine dioxide (ClO_2) has been recognized as an attractive alternative. Among its attributes are the lack of formation of trihalomethane and chloramine derivatives, its oxidative properties throughout a broad pH range, and its high solubility in water. Studies of the efficacy of ClO_2 against *C. parvum* oocysts have been reported in the peer-reviewed literature, however these studies primarily focused on development of CT values for use in the drinking water sector (in which a 2-log_{10} reduction of oocysts is required), used a non-ideal assay viability method such as excystation, analyzed the sequential effects of ClO_2 with other disinfectants, or varied in the disinfection kinetics model applied to the data.

Materials and Methods

25°C environmental chamber

Reaction flasks:
1. 5 mg/L ClO_2
2. 5 mg/L ClO_2 + 2 mg/L FC
3. 20 mg/L FC control
4. Oxidant demand-free water control

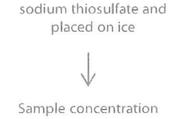
\+ *C. parvum* oocysts (final conc. 10^5 oocysts/mL)



Samples quenched with sodium thiosulfate and placed on ice

Measurement of free chlorine, chlorine dioxide, pH, and ORP

Sample concentration via centrifugation

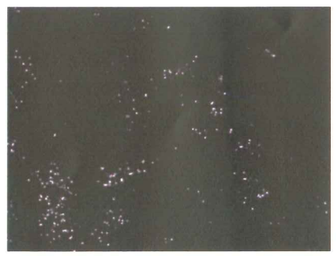


Concentrated samples inoculated onto MDCK cell layers

Incubation at 37°C with 5% CO_2 for 48-60 hours

Fixation and 1° and 2° antibody labeling

250X immunofluorescence microscopy



Cryptosporidium meronts and gamonts defined by size, shape, and fluorescent labeling[3]

***C. parvum* oocysts:** Waterborne, Inc., New Orleans, LA, originating from a bovine source in Iowa (Harley Moon isolate), stored at 4°C in a saline solution containing penicillin and streptomycin for ≤3 months.

Madin-Darby canine kidney (MDCK) cells: lines were routinely passaged and were inoculated onto cover class-bottom culture chambers (Nunc Lab-Tek, Rochester, NY) for infectivity assays using modified Dulbeco's Mimimum Essential Media (DMEM).

Test Water Preparation: All assays were conducted using buffered (pH 7.5) sterile oxidant demand-free (ODF) test water and glassware prepared as previously described[1]. Test water was autoclaved prior to experiments. A concentrated ClO_2 solution was prepared using Aseptrol®, a stabilized ClO_2 product (BASF).

Data Analysis: The efficiency factor Hom (EFH) model[2] was used to calculate CT values for log_{10} inactivation. This model incorporates both the reduction of *C. parvum* living stages and ClO_2 decay over time using a first-order kinetic equation. Inactivation curves were created using Microsoft Excel in order to assess survival under ClO_2 conditions both in the presence and absence of 2 mg/L free chlorine).

Results

Table 1: CT values (mg·L/min) for 5 mg/L ClO_2

	1 log_{10}	2 log_{10}	3 log_{10}
Exp #1	232	388	564
Exp #2	322	420	473
Exp #3	387	546	666
Exp #4	444	639	784
Average ± std dev	346 ±91	498 ±116	622 ±134

Table 2: CT values (mg·L/min) for 5 mg/L ClO_2 + 2 mg/L FC

	1 log_{10}	2 log_{10}	3 log_{10}
Exp #1	300	378	422
Exp #2	254	363	453
Exp #3	194	314	428
Exp #4	265	465	654
Average ± std dev	253 ±44	380 ±63	489 ±111

- According to the EFH model, CT values for a 3-log_{10} reduction of *C. parvum*:
  - 622 mg·min/L for 5 mg/L ClO_2
  - 489 mg·min/L for 5 mg/L ClO_2 + 2 mg/L free chlorine
- 20 mg/L free chlorine control 1-log_{10} CT values were:
  - 3490 mg·min/L for 1 log_{10} reduction
    [Shields et al (2008)[4]: 6900 mg·min/L for 2 log_{10} reduction]
  - ~10x – 15x higher CT value compared with 5 mg/L ClO_2
- Average ORP (mV) measurements:

Disinfectant Condition	0 min	180 min
5 mg/L ClO_2	720	588‡
5 mg/L ClO_2 + 2 mg/L FC	711	590‡
20 mg/L FC	754	754

‡Average ClO_2 concentration at 180 min was 1.5 mg/L

Conclusion and Discussion

- Peer-reviewed studies that report ClO_2 CT values for *C. parvum* vary both in methodology and results. This study expands upon previous work by using a quantitative cell culture infectivity assay and incorporating ClO_2 decay into the estimated CT values.
- **For a 99.9% inactivation of *C. parvum* oocysts, using 5 mg/L ClO_2 (124 minutes) or 5 mg/L ClO_2 + 2 mg/L free chlorine (98 minutes) would substantially reduce the time required for swimming pool closure compared to existing CDC recommendations using 20 mg/L free chlorine (765 minutes)**
- **ClO_2 can be hazardous if used improperly and safety training is highly recommended prior to use.**

References:
[1]APHA. 1995. *Standard Methods for the Examination of Water and Wastewater*. Washington, DC, USA
[2]Haas CN, Joffre J. 1994. *Environ Sci Technol*. **28**(7):1367-1369
[3]Arrowood MJ. 2002. *Clin Microbiol Rev*. **15**(3):390-400
[4]Shields JM, Hill VR, Arrowood MJ, Beach MJ. 2008. *J Water Health*. **6**(4): 513-520

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