Science of the Total Environment 599–600 (2017) 533–539

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Comparing peracetic acid and hypochlorite for disinfection of combined sewer overflows: Effects of suspended-solids and pH

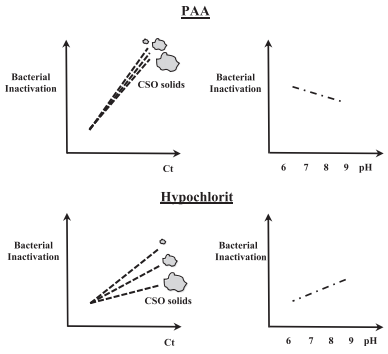
M. McFadden [a], J. Loconsole [a], A.J. Schockling [a], R. Nerenberg [a], J.P. Pavissich [a,b,*]

[a] Department of Civil and Environmental Engineering and Earth Sciences, University of Notre Dame, IN 46556, USA
[b] Facultad de Ingeniería y Ciencias, Universidad Adolfo Ibáñez, Santiago, Chile

HIGHLIGHTS

- The efficiencies of peracetic acid (PAA) and hypochlorite were tested for different wastewater particle sizes.
- Hypochlorite's efficiency decreased with larger particles, while PAA was less sensitive to size.
- PAA was more effective at lower pH, while hypochlorite was more effective at higher pH.
- PAA and hypochlorite appear to have similar cell inactivation mechanisms.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 9 February 2017
Received in revised form 22 April 2017
Accepted 23 April 2017
Available online 6 May 2017

Editor: Simon Pollard

Keywords:
PAA
Chlorine
Combined sewer overflows
Suspended solids
Disinfection

ABSTRACT

Peracetic acid (PAA) is an alternative disinfectant that may be effective for combined sewer overflow (CSO) disinfection, but little is known about the effect of particle size on PAA disinfection efficiency. In this work, PAA and hypochlorite were compared as disinfectants, with a focus on the effect of wastewater particles. Inactivation experiments were conducted on suspended cultures of *Escherichia coli* and wastewater suspended solids. Tested size fractions included particle diameters < 10 μm, < 100 μm, and raw wastewater. Chlorine disinfection efficiency decreased with increasing solids size. However, solids size had little effect on PAA disinfection. The PAA disinfection efficiency decreased at pH values above 7.5. Live/dead staining revealed that PAA disinfection leaves most cells in a viable but non-culturable condition. Fourier transform infrared spectroscopy (FTIR) analyses suggests that PAA and hypochlorite may inactivate *E. coli* bacteria by similar mechanisms.

1. Introduction

Combined sewer overflows (CSOs) are a common problem in the United States and throughout the world (NRDC, 2011). In the United States, the EPA has made CSO abatement a priority (USEPA, 1999; USEPA, 1994). However, complete elimination of CSOs is often

* Corresponding author.
E-mail address: juan.pavissich@uai.cl (J.P. Pavissich).

http://dx.doi.org/10.1016/j.scitotenv.2017.04.179
0048-9697/

economically prohibitive. Regulations increasingly require disinfection of the occasional CSO discharges, which contains high total suspended solids (TSS) and potentially has large particle sizes. The ideal disinfectant would achieve at least 4-log reduction (99.99% removal) of bacterial concentrations with contact times of 15 to 30 min, and avoid the formation of toxic by-products (Coyle et al., 2014).

A popular option for CSOs is chlorine-based disinfectants, which are effective against bacteria, viruses, and protozoan cysts, and are commonly used for disinfection of wastewater final effluents (De Luca et al., 2008; Lazarova et al., 1998; Veschetti et al., 2003). However, chlorine derivatives often have high rates of decay during storage, are sensitive to changes in pH, can form toxic disinfection by-products upon reaction with organics, and require quenching to remove residual disinfectants prior to discharge. These characteristics make them less desirable for CSOs, which are intermittent and more variable in volume and composition. Also, CSO discharges often include higher amounts of suspended solids, which may shelter pathogens and impact the chlorine disinfection efficiency (Rossi et al., 2007).

Peracetic acid (PAA), a non-conventional disinfectant, has shown potential as an environmentally-friendly and cost-effective alternative for CSO disinfection (Chhetri et al., 2014, Coyle et al., 2014; Kitis, 2004; Luukkonen and Pehkonen, 2017). PAA is a relatively strong oxidant, and is highly effective for disinfection of bacteria; it has also demonstrated some antiviral properties (Baldry and French, 1989a; Baldry et al., 1991; Koivunen and Heinonen-Tanski, 2005).

PAA is commercially available in a stable, quaternary equilibrium mixture of acetic acid, hydrogen peroxide, peracetic acid, and water (Kitis, 2004):

$$CH_3COOH + H_2O_2 \leftrightarrow CH_3CO_3H + H_2O$$

As a weak acid, the dissociation constant, pKa, of PAA is 8.2 (Coyle et al., 2014; Santoro et al., 2005). When the pH is below 8.2, PAA is thought to spontaneously decompose into reactive oxygen, which can disrupt sulfhydryl (—SH) and sulfur (S—S) bonds within enzymes and proteins in cell membranes. As a result, transport across the cell membrane is hindered and cellular function is impaired (Gehr et al., 2003; Lefevre et al., 1992). Other oxidizing agents, including chlorine derivatives, have disinfection mechanisms similar to those proposed by Lefevre, involving disruption of cell membranes by reactive oxygen (Kitis, 2004). However, the mechanism of disinfection by PAA remains uncertain, with some studies suggesting that PAA releases hydroxyl radicals, which readily oxidize proteins and lipids to compromise bacterial cell walls and to denature cellular DNA (Coyle et al., 2014; Koivunen and Heinonen-Tanski, 2005; Lubello et al., 2002).

A major advantage of PAA over chlorine-based disinfectants is that it reacts and decomposes quickly, possibly eliminating the need for quenching prior to discharge and potentially requiring shorter contact times (Coyle et al., 2014; De Luca et al., 2008; Kitis, 2004). It is believed to produce little to no toxic by-products upon reaction with wastewater or natural organic matter (Baldry and Fraser, 1988; Monarca et al., 2002).

PAA has been demonstrated as an advantageous alternative for treating effluent wastewaters with low doses and short contact times, and could feasibly be used to treat CSOs (Baldry et al., 1995; De Luca et al., 2008; Rossi et al., 2007; Wagner et al., 2002). However, past PAA research has mainly addressed the disinfection of pure cultures of bacteria, or wastewater effluents with few or very small particles.

Several bench-scale studies have addressed PAA disinfection of wastewater, but researchers have found that the effective PAA dosage and contact times vary widely based on water quality (Baldry and French, 1989b; Coyle et al., 2014; Sanchez-Ruiz et al., 1995). Also, a major unknown is the effect of wastewater particle quantity and size on PAA disinfection efficiency (Falsanisi et al., 2008). While it has been demonstrated that high disinfection efficiency can be achieved with PAA in wastewater containing TSS up to 100 mg/L, suspended solids in CSOs can range from 10 to over 1000 mg/L (Lefevre et al., 1992; USEPA, 1999). The particle size of solids in CSOs, ranging from a few microns to hundreds of microns, may allow larger solids to shelter pathogens from disinfection. Also, disinfectants may react with organics in the solids, limiting the available biocide concentration (Coyle et al., 2014).

Previous work on PAA disinfection has shown that disinfection efficiency decreases with increasing TSS (Kitis, 2004). Studies have also looked into bacterial sheltering and tailing phenomena produced by suspended solids during UV and chlorine disinfection (Liang et al., 2013; Yong et al., 2009). Tailing occurs when a group of microorganisms survives disinfection and the population stabilizes with extended disinfection time (Liang et al., 2013). Despite previous studies into the TSS effect, little has been done to correlate suspended solids sizes with disinfection efficiency and to study the direct effect of disinfection on particles using microscopy (Falsanisi et al., 2008). The solids present in CSOs may provide a diffusive barrier that shelters bacteria from disinfection.

The objective of this study was to determine the effect of suspended solids on PAA disinfection efficiency, and to compare PAA disinfection to disinfection by free chlorine as hypochlorite, a conventional chlorine-based disinfectant. Tests were carried out on a pure culture of *E. coli* and on wastewater solids.

2. Materials and methods

2.1. Disinfection of E. coli

Initial disinfection tests were performed on suspended *E. coli* cultures in a synthetic medium. *E. coli* were grown to exponential phase on 2% Luria Bertani broth (LB) in batch culture. Cells were centrifuged for 5 min at 6,700 *g*, washed, and re-suspended with physiological saline solution (0.85% NaCl in deionized water) to a cell count of approximately 10^8 to 10^9 CFU/mL. A 1 mL aliquot was inoculated into tubes containing 9 mL of PAA in saline solution, with concentrations ranging from 0.5 ppm to 2.0 ppm as PAA. The tubes were placed on a shaker at ambient temperature for a 10-min contact time, which has been reported as an effective contact time for PAA disinfection (Kitis, 2004). Immediately after disinfection, samples were centrifuged, washed in saline solution, plated onto Coliscan Easygel® plates (Micrology Laboratories, Goshen, IN), and incubated overnight at 36 °C. Colonies were quantified using the dilution to extinction method. The same procedure was used to assess sodium hypochlorite disinfection, using concentrations of 2 to 10 ppm as Cl.

2.2. PAA and chlorine quantification

A 39% PAA solution (Sigma-Aldrich, St. Louis, MO, USA) and a Hypochlorite solution (Clorox, Oakland, CA, USA) were diluted to working concentrations of 10 ppm to 100 ppm. A spectrophotometric method was employed to validate initial PAA and hypochlorite concentrations, and to later quantify residual disinfectant (Harvey and Howarth, 2010). This method is based on the colorimetric reaction of iodide ion and a N,N-diethyl-*p*-phenylenediamine (DPD) indicator, and can be easily implemented using commercially-available kits for measuring total chlorine, such as the Hach DPD total chlorine test (Hach Company, Loveland, CO). Samples were prepared using the total chlorine kit and measured at 530 nm, per the manufacturer's instructions. A simple calculation was performed to convert the test result of PAA samples (given as ppm of chlorine) to ppm of PAA, based on the molecular weight ratio of PAA to Cl_2 (76:71, or 1.07).

2.3. Characterization of wastewater primary influent

Experiments were carried out on wastewater samples collected after preliminary treatment (primary clarifier influent) from a local municipal wastewater treatment plant (Mishawaka Wastewater Treatment

Plant, Mishawaka, IN), to mimic the TSS and bacterial content of a CSO. The wastewater had a pH of 7.5. Serial filtration separated the wastewater into the following fractions: unfiltered "raw" wastewater, wastewater with TSS > 100 μm, and wastewater with TSS > 10 μm. The larger fractions were normalized in terms of TSS concentrations by diluting with filtered primary effluent wastewater (0.2 μm filter). TSS was measured for each fraction using the Standard Methods for the Examination of Water and Wastewater (APHA et al., 2005).

2.4. Disinfection of primary influent

Disinfection tests were performed on each wastewater size fraction to investigate the effect of suspended solids size on disinfection of CSO-like water. As a control, a 5-mL sample of each fraction was sonicated and plated prior to disinfection to assess the total bacteria count "sheltered" in the solids. The sonication dose applied was 50 J/mL, which was determined to be the optimal dose for releasing the greatest number of viable cells from solids. For disinfection tests, 10-mL triplicate samples of each normalized fraction containing a concentration of 0.5 to 1.5 ppm PAA were tested. The tubes were placed horizontally on an orbital shaker at ambient temperature for a 10-min disinfection contact time. Disinfected samples were centrifuged and the pellets were resuspended in physiological serum and plated onto Coliscan® plates, following the dilution to extinction method. Plates were incubated overnight at 36 °C, and the colonies were quantified using a stereomicroscope. This procedure was repeated using hypochlorite solutions of concentration 2 to 4 ppm.

2.5. pH tests

To determine the effect of pH on disinfection tests, the wastewater fractions were each adjusted to a pH of 8.5 or 6.5, using either NaOH or HCl, as appropriate. Disinfection tests were conducted as previously described with the pH-adjusted wastewater, using concentrations of 0.5 ppm PAA or 2.0 ppm hypochlorite for a 10-min disinfection contact time.

2.6. Microbial enumeration

Pre- and post-disinfection plating was performed to enumerate culturable bacteria. Serial dilutions were plated on Coliscan® plates. The plates were incubated at 36 °C overnight and colonies were counted using a stereomicroscope. The bacterial inactivation (I) was expressed as the viable counts log reduction after a disinfection test according to the following equation:

$$I = \log\frac{N}{N_0} \quad (1)$$

where N and N_0 are the colony forming units present after disinfection and the initial colony count prior to disinfection, respectively.

2.7. Microscopy

Cell viability was assessed using three different staining methods. A FilmTracer™ Live/Dead® Biofilm Viability Kit (BacLight; InVitrogen by Life Technologies, Carlsbad, CA) was utilized to prepare a two-color fluorescence assay of bacterial viability based on membrane integrity. In this kit, the SYTO®9 nucleic acid stain diffuses through all bacterial membranes to stain the cells green. Propidium iodide (PI) penetrates cells with compromised membranes, and the combination of the two stains produces red fluorescing cells. The wastewater samples were combined with the stain in a 1:5 ratio (sample:stain) and were allowed to incubate for 10 min prior to observation with a fluorescent imaging microscope.

Samples were also subjected to staining by 5-Cyano-2,3-ditolyl tetrazolium chloride (CTC), which is reduced by actively-respiring cells to CTF, the red-fluorescing formazoan crystals of CTC. Wastewater samples were combined with a 4 mM CTC solution and incubated for 10 min prior to microscopy.

DAPI nucleic acid stain was used as a counterstain to CTC in order to indicate total cells present. The 1 mg/mL solution stained nucleoids with blue fluorescent stain. This double-staining procedure allowed for the identification of viable cells among the entire cell population.

For both staining procedures, a fluorescent imaging microscope (Nikon Eclipse 90i), equipped with a 40× oil immersion objective, was utilized to capture images of fluorescing cells. Images were processed using ImageJ software (http://rsb.info.nih.gov/ij/).

2.8. FTIR

Fourier transform infrared spectroscopy (FTIR) was employed to further investigate the mechanisms of bacterial disinfection by PAA and hypochlorite, as shown elsewhere (Xue et al., 2012). The current study analyzed the disinfection of a pure *E. coli* culture. To obtain a large biomass sample, 1 mL of a culture grown overnight, was centrifuged (5 min at 6700 g) and washed with saline solution. The resuspended *E. coli* pellet was disinfected as previously described using either PAA or hypochlorite. Immediately after disinfection, samples were passed quickly through a 0.2 μm Millipore glass fiber filter (Darmstadt, Germany), which collected the disinfected cells. Filters were dried at room temperature and analyzed using an absorption spectrum from 4000 cm^{-1} to 400 cm^{-1} in a Bruker Tensor 27 FTIR using the OPUS Data Collection software.

3. Results and discussion

3.1. Disinfection kinetics of E. coli

To demonstrate the relative susceptibility of the pure *E. coli* culture to disinfection by PAA or hypochlorite, inactivation curves were plotted as the log of the fraction of culturable bacteria (log (N/N_0)) versus Ct (disinfectant concentration × contact time) (Fig. 1). The Ct (mg-min L^{-1}) was changed by varying the disinfectant concentration for the same 10-min contact time. Results indicated that, after a given "lag Ct" of inactivity, *E. coli* was susceptible to rapid disinfection by both PAA and hypochlorite.

To obtain a more quantitative evaluation of the non-log-linear survival plot of *E. coli* inactivation, the Geeraerd and Van Impe inactivation model-fitting tool was used (GInaFiT, Version 1.5; KU Leuven, Belgium). The applied model used a log-linear model with shoulder and tailing

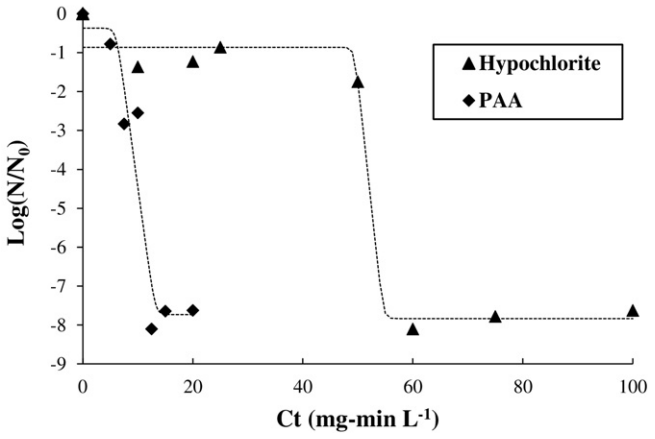


Fig. 1. Representative inactivation kinetics by PAA and hypochlorite for *E. coli*. pH = 7.5, log-linear fit model.

(Eq. (1); (Geeraerd et al., 2005). The shoulder (S_1) accounts for the lag time before the bacteria respond to disinfection, while the tailing (N_{res}) accounts for the number of resistant colonies which survive disinfection. The specific inactivation rate (k_{max}) indicates the rates of inactivation.

$$N_t = (N_0 - N_{res})e^{(-k_{max}t)} \frac{e^{(k_{max}S_1)}}{1 + (e^{(k_{max}S_1)} - 1) \times e^{(-k_{max}t)}} + N_{res} \quad (2)$$

While the rate of inactivation, k_{max}, was higher for hypochlorite, the disinfection lag, S_1, was approximately 8.2 times shorter for PAA (Table 1). This indicates that although hypochlorite acts at a slightly higher rate to disinfect colonies, it requires a much longer CT before efficient disinfection can be achieved. Overall, it appears that PAA, with its shorter lag, is the more efficient disinfectant for a pure *E. coli* culture.

Previous studies of PAA inactivation have observed similar trends of disinfection lag with *E. coli* in the presence of low doses of PAA (Rossi et al., 2007; Santoro et al., 2005). This trend has been attributed to initial resistance to PAA imparted by the cell membrane, which delays PAA inactivation effects (Rossi et al., 2007). It has also been shown that the reactivity of capsular extracellular polymeric substances (EPS), which is produced by many microorganisms, including *E. coli*, retards disinfection (Xue et al., 2012). Xue et al. (2012) found that capsular EPS provides multiple forms of protection against various disinfectants, as it reduces membrane permeabilization by acting as a disinfectant consumer or limits access to reactive sites on the cellular membrane. The mechanism of protection is dependent upon the disinfectant species, as EPS exhibits different levels of reactivity with different disinfectants (Xue et al., 2012). Though it has not yet been studied, EPS may provide better protection against hypochlorite than PAA.

The differences in disinfection mechanism between PAA and hypochlorite may affect their disinfection efficiency. Active oxygen produced during PAA reaction affects sulfhydryl and sulfur bonds in cell membrane and proteins, whereas hypochlorite is nonselective and rapidly reacts with both organic and nonorganic compounds (Baldry and Fraser, 1988; Jolivet-Gougeon et al., 2006; Lefevre et al., 1992; Xue et al., 2012). It is possible that PAA targets the EPS capsule, which is composed of polysaccharides, proteins, and lipids. This would provide a greater disinfection efficiency for the *E. coli*.

3.2. Disinfection kinetics of primary influent

The principal experiments conducted in this study utilized primary influent from a local treatment plant in order to investigate the effect of suspended solids size on disinfection of CSO-like water. Experimental results plotted in Fig. 2 illustrate PAA and hypochlorite inactivation kinetics for various TSS size fractions as a function of different Ct values. The Ct was obtained by using different PAA or hypochlorite concentrations for a 10-min contact time.

Results were consistent with other studies, which demonstrated that bacterial sheltering by coarse particles has the potential to reduce disinfection efficiency (Falsanisi et al., 2008; Koivunen and Heinonen-Tanski, 2005). The shielding phenomenon was observed for both PAA and hypochlorite, where the smallest solids (<10 μm) achieved more efficient disinfection than larger solids. However, the differences in disinfection efficiency were more significant for hypochlorite, where there was a 2–3 log difference in inactivation among particle-associated bacteria present in solids of various sizes.

Table 1
E. coli disinfection kinetic parameters.

	S_1 (mg-min L^{-1})	k_{max} (min^{-1})
PAA	6.03 ± 2.21	2.37 ± 1.04
Hypochlorite	49.38 ± 3.46	3.03 ± 1.68

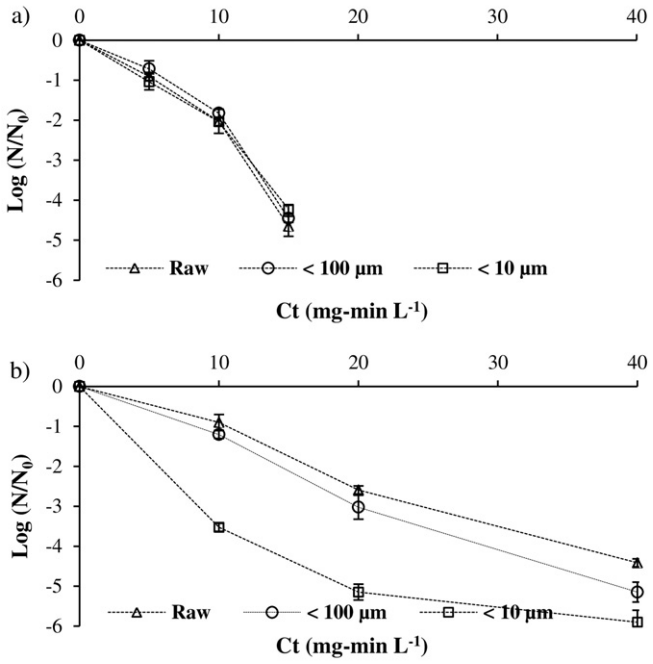


Fig. 2. Representative inactivation kinetics for total coliforms in primary effluent wastewater with different suspended solids size fractions using a) PAA and b) hypochlorite, pH = 7.5 (error bars ±1 standard deviation, $N = 4$).

The difference between PAA and hypochlorite disinfection curves may be attributed to their specific disinfection mechanisms. Reactive oxygen produced by PAA affects bonds in cell membranes and proteins, whereas hypochlorite is nonselective and rapidly reacts with both organic and inorganic compounds (Lefevre et al., 1992; Xue et al., 2012). Previous studies have demonstrated that PAA is not significantly influenced by variability in the composition of wastewater, whereas hypochlorite is highly sensitive to changes in organic matter, which compromises its disinfection efficiency (Baldry et al., 1991; Baldry et al., 1995; De Luca et al., 2008; Lazarova et al., 1998).

It was also observed that none of the disinfection curves obtained in this experiment exhibit the large "shoulders" shown in the tests with suspended *E. coli* cultures (Fig. 1). This observation indicates that cells in wastewater are more susceptible to immediate disinfection than the cultured *E. coli*, which is known to form a protective EPS capsule.

The tailing trend that was previously observed with the inactivation of a suspended *E. coli* culture is seen with hypochlorite inactivation of particle-associated total fecal coliforms. Tailing is an indicator of decreasing inactivation rate, and occurs at high Ct values due to highly resistant bacterial cultures (Koivunen and Heinonen-Tanski, 2005). Some of the tailing observed with hypochlorite disinfection could be due to bacterial sheltering by coarse particles or microbial clumping (Koivunen and Heinonen-Tanski, 2005). It is clear that hypochlorite disinfection efficiency is reduced for larger particles, and cells located at the middle of dense solids could be more difficult to disinfect. This indicates that a higher concentration of hypochlorite would be necessary in order to achieve efficient disinfection of particle-associated bacteria.

3.3. pH tests

Because CSOs are variable in composition, their pH can fluctuate (USEPA, 1995). To understand how the variation of pH might affect treatment by PAA and hypochlorite, wastewater fractions were adjusted to a pH of 6.5 and 8.5 prior to disinfection. It was observed that the disinfection efficiency of PAA increased in more acidic conditions (Fig. 3). On the other hand, hypochlorite disinfection efficiency significantly increased in more alkaline conditions.

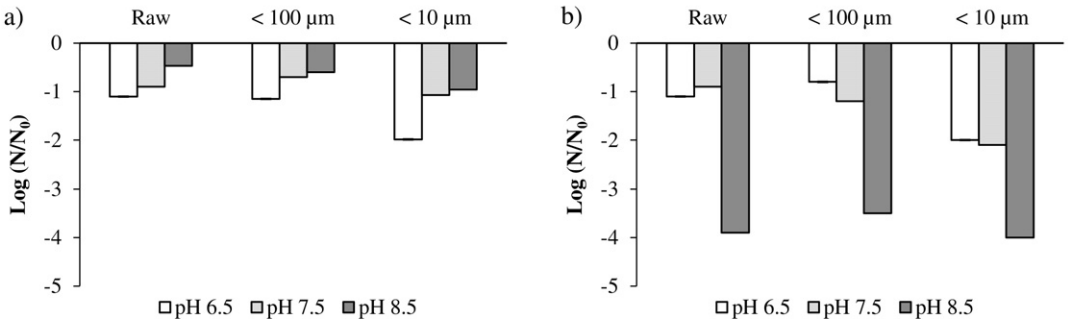


Fig. 3. Effect of pH on total coliform inactivation in primary effluent. a) PAA, Ct = 5 mg-min L^{-1} and, b) hypochlorite, Ct = 10 mg-min L^{-1} (error bars ± 1 standard deviation, $N = 3$, not visible on the scale).

Fig. 3 shows the effect of particle size for one Ct value. Consistent with Fig. 2, PAA was not significantly affected by particle size for pH values of 7.5 and 8.5. However, at a pH of 6.5, the disinfection efficiency was nearly one log higher for the smaller particle size.

At a pH value below PAA's pKa of 8.2, the un-dissociated form of PAA (CH_3CO_3H) spontaneously decomposes to produce reactive oxygen, reaching a maximum decomposition rate at pH 8.2 (Colgan and Gehr, 2001; Kitis, 2004; Yuan et al., 1997). When the pH of solution rises above 8.2, the ionized form of PAA ($CH_3CO_3^-$) dominates and hydrolyzes to produce peracetate ions and acetate ions (Coyle et al., 2014). The rate of hydrolysis increases with increasing pH. Because peracetate and acetate ions have minimal disinfecting capacity, the disinfection efficiency of PAA is greatly reduced when used in solution with a pH >8.2 (Baldry and French, 1989a; Sanchez-Ruiz et al., 1995; Yuan et al., 1997). Baldry and French (1989a) also found that neutral or mildly acidic conditions promoted greater PAA efficiency against fecal coliforms than alkaline conditions. It should also be noted that smaller particles allowed for greater disinfection efficiency with PAA. This trend was more significant at acidic pH than in the disinfection experiments of primary effluent at neutral pH and same Ct, as shown above.

For hypochlorite disinfection, there was no observable trend with solids size. Moreover, all of the samples of pH 8.5 reached full disinfection, while the samples of pH 6.5 were less-efficiently disinfected, which was unexpected. In aqueous solutions an equilibrium is formed between hypochlorous acid (HOCl) and hypochlorite anion (OCl^-), with a pKa value of 7.5. HOCl and OCl^- together form free chlorine. Both are non-specific oxidants that readily react with dissolved and particulate organic matter. Typically, hypochlorite disinfection is more active below a pH of 7.5, when chlorine is mainly present in the more reactive unionized state HOCl. HOCl is much more reactive to cells than OCl^-, and reaction rates tend to increase with decreasing pH (McDonnell and Russell, 1999). HOCl is also more prone to penetrate bacterial cell membranes to attack DNA and proteins, because its molecular structure is similar to water and also lacks electrical charge (Del Carpio-Perochena et al., 2015). However, for bacteria contained in a particulate organic matrix, diffusion of active chlorine can be overcome by its fast consumption due to reaction with organic compounds, reducing its biocidal effect (Stewart et al., 2001). According to the reaction–diffusion theory, a less reactive biocide should penetrate organic material more effectively (Chen and Stewart, 1996). This can explain the observed trend at pH above 7.5, where the OCl^- ions predominate and the highest disinfection efficiency was observed. On the other hand, at alkaline conditions HOCl can dissolve a greater quantity of particulate organic matter (Del Carpio-Perochena et al., 2015). We hypothesize that a higher dissolution of particulate organic matter may expose bacterial cells sheltered in particles, and make them more susceptible to disinfection.

3.4. Microscopy

Previous studies have shown that fluorescent staining, coupled with plate count methods, can be used to investigate disinfectant efficiency in suspended cultures by enumerating both viable and total counts of bacteria after disinfection (Xue et al., 2012). Cells are “alive” when

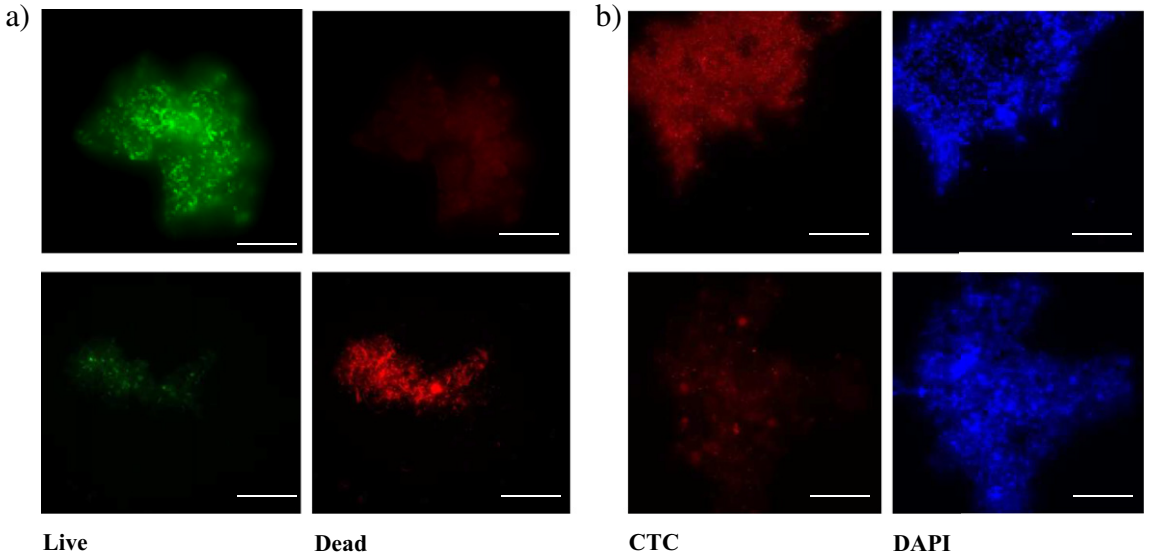


Fig. 4. Fluorescent microscopy of solid particles before (top) and after (down) treatment with PAA (Ct = 10 mg-min L^{-1}), showing (a) live and dead cells, and (b) respiring and total cells. Scale bar = 100 μm.

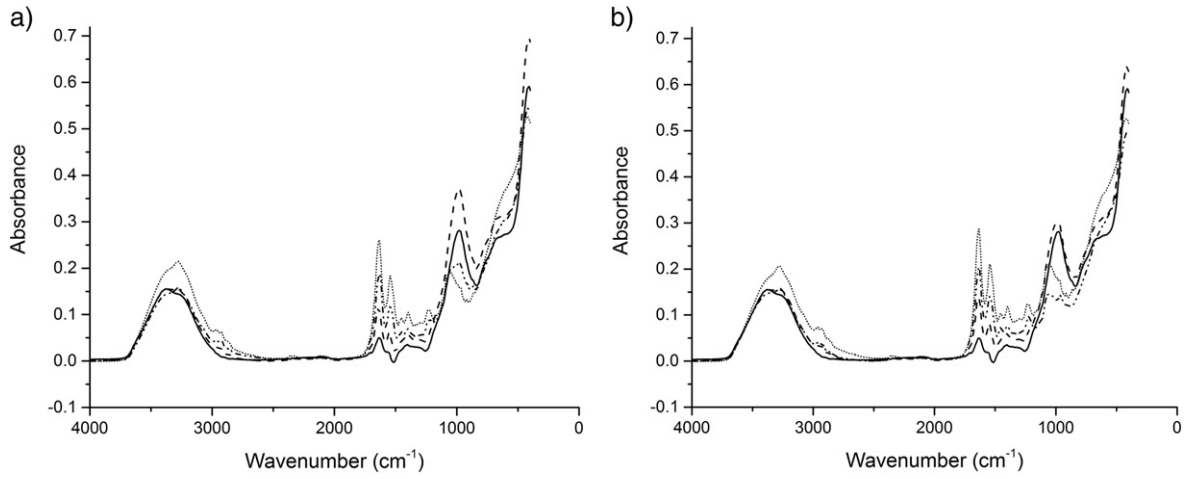


Fig. 5. FTIR absorbance spectra of *E. coli* disinfection. a) PAA, — t_0, ···· 0.5 ppm, - - - 5 ppm, — · 25 ppm. b) Hypochlorite, — t_0, ···· 2 ppm, - - - 20 ppm, — · 100 ppm.

they exhibit an intact cell membrane or respiratory activity. Fig. 4A shows representative microscopy images of LIVE/DEAD staining observations with disinfection of particle-associated bacteria on suspended solids. As expected, staining indicated that most cells were alive (i.e., their cell walls had not been compromised) prior to disinfection. However, the same trend was observed post-disinfection, where, unexpectedly, particles of all fractions maintained similar areas with viable cells after disinfection. In order to further investigate this phenomenon, CTC, a metabolic stain, was employed to provide an indication of actively respiring cells (Fig. 4B). DAPI, a nucleic acid stain, was used as a counterstain to CTC in order to show the total cells present. With the counterstain, it was observed that the number of respiring cells appeared similar to the total cells present, reinforcing previous results that indicated that cells remained viable post-disinfection.

Thus, PAA impacts the culturability of disinfected bacteria, but not necessarily their viability. Similar results have previously been found for chlorine-based disinfectants (Oliver et al., 2005).

3.5. FTIR

FTIR was used to investigate the mechanism of disinfection by PAA and hypochlorite on *E. coli* cells. FTIR measures vibrational transitions by light absorption, and generates absorbance peaks to indicate the various frequencies and intensities of light absorbed by the sample. FTIR can be utilized to probe the chemical composition of bacterial cells and to observe changes caused by disinfectants at the functional group level (Holt et al., 1995; Xue et al., 2012). Xue et al. (2012) used FTIR to identify spectral bands that were altered during disinfection of three bacterial species by monochloramine and chlorine. Based on the results of that study, it was expected that the absorption bands of the cells disinfected by PAA and hypochlorite would show alteration at the 3700–2700 and 1800–900 cm^{-1} wavelengths, indicating specific cellular functional group changes produced by chemical reaction with the disinfectants.

Unaltered and first-derivative transformed spectra for PAA- and hypochlorite-treated *E. coli* samples are shown in Fig. 5 and Fig. S1, respectively. The unaltered spectra of the *E. coli* samples, both prior to disinfection and after disinfection by PAA and hypochlorite, respectively, appear to be very similar to the patterns obtained by Xue et al. (2012) (Fig. 5). The first-derivative transformed spectra show significant differences in the absorbance peaks for the disinfected samples compared to *E. coli* samples taken prior to disinfection (Fig. S1). Based on the characteristic band assignment summarized by Xue et al. (2012), the spectral bands primarily impacted were the N—H deformation and C—N stretching of amide, at the 1620–1590 and 1550–1510 cm^{-1} ranges. Less significant absorbance changes were observed for peaks associated to the C—H and O—H deformation vibrations of carbohydrates, at the 1460–1350 cm^{-1} range (Fig. S1). These results suggest that PAA and hypochlorite may a higher interaction with protein than polysaccharides groups in *E. coli*. The first-derivative transformed absorption peaks from the PAA- and hypochlorite-disinfected samples are in phase with one another, suggesting that the overall mechanism of disinfection of both PAA and hypochlorite may target the same functional groups (Fig. S1). The FTIR spectra were also transformed by taking the second derivative, with the goal of comparing the second derivative transformed absorption spectra to those obtained by Xue et al. (2012). However, the intensities of the second derivative peaks were very low, and no obvious trends were observed (data not shown).

4. Conclusions

This study provides a direct comparison of two disinfectants, PAA and hypochlorite, on CSO-like wastewater and for a water matrix containing a pure *E. coli* culture. While hypochlorite reached a higher maximum inactivation rate for the pure *E. coli* culture, hypochlorite required much higher CT values than PAA before effective disinfection could begin.

Inactivation of CSO-like water containing suspended solids was studied at pH values of 6.5, 7.5, and 8.5. In these tests, disinfection by PAA was more efficient than disinfection by hypochlorite for all solids size fractions. In fact, particle size had little effect on PAA disinfection efficiency, while it had a much greater effect on hypochlorite disinfection. However, PAA disinfection efficiency decreased at pH values above 7.5. Similar to hypochlorite, disinfection by PAA left cells in a viable but not culturable state.

Results from FTIR showed changes in the chemical composition of *E. coli* cells, and suggested that PAA compromises cells in a similar way to hypochlorite, targeting protein and polysaccharides functional groups. Further data analysis is necessary in order to determine how the specific chemical nature of the bacteria is changed upon disinfection.

Overall, this research suggests that PAA is an effective disinfectant for CSOs. These results may also have important practical implications for utilities considering PAA disinfection. It also may have implications for the disinfection of biofilms, or for disinfection in cases where cells are embedded in a particulate organic matrix.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2017.04.179.

Acknowledgements

Funding was provided by the Ganey Foundation for Community-Based Research and the Clare Boothe Luce Research Fellowship. The

FTIR analyses were conducted at the Center for Environmental Science and Technology (CEST) at University of Notre Dame.

References

APHA, AWWA, WEF, 2005. Standard Methods for the Examination of Water and Wastewater. 21st ed. (Washington, DC).

Baldry, M.G.C., Fraser, J.A.L., 1988. Disinfection with peroxygens. Crit. Rep. Appl. Chem. 22, 91–116.

Baldry, M.G.C., French, M.S., 1989a. Activity of peracetic acid against sewage indicator organisms. Water Sci. Technol. 21, 1747–1749.

Baldry, M.G.C., French, M.S., 1989b. Disinfection of sewage effluent with peracetic acid. Water Sci. Technol. 21, 203–206.

Baldry, M.G.C., French, M.S., Slater, D., 1991. The activity of peracetic acid on sewage indicator bacteria and viruses. Water Sci. Technol. 24, 353–357.

Baldry, M.G.C., Cavadore, A., French, M.S., Massa, G., Rodrigues, L.M., Schirch, P.F.T., Threadgold, T.L., 1995. Effluent disinfection in warm climates with peracetic acid. Water Sci. Technol. 31, 161–164.

Chen, X., Stewart, P.S., 1996. Chlorine penetration into artificial biofilm is limited by a reaction–diffusion interaction. Environ. Sci. Technol. 30, 2078–2083.

Chhetri, R.K., Thornberg, D., Berner, J., Gramstad, R., Ojstedt, U., Sharma, A.K., Andersen, H.R., 2014. Chemical disinfection of combined sewer overflow waters using performic acid or peracetic acids. Sci. Total Environ. 490, 1065–1072.

Colgan, S., Gehr, R., 2001. Disinfection. Peracetic acid gains favor as an effective, environmentally benign disinfection alternative for municipal wastewater treatment applications. Water Environ. Technol. 13, 29–33.

Coyle, E.E., Ormsbee, L.E., Brion, G.M., 2014. Peracetic acid as an alternative disinfection technology for wet weather flows. Water Environ. Res. 86, 687–697.

De Luca, G., Sacchetti, R., Zanetti, F., Leoni, E., 2008. Comparative study on the efficiency of peracetic acid and chlorine dioxide at low does in the disinfection of urban wastewater. Ann. Agric. Environ. Med. 15, 217–224.

Del Carpio-Perochena, A., Bramante, C.M., De Andrade, F.B., Maliza, A.G.A., Cavenago, B.C., Marciano, M.A., Amoroso-Silva, P., Duarte, M.H., 2015. Antibacterial and dissolution ability of sodium hypochlorite in different pHs on multi-species biofilms. Clin. Oral Invest. 19, 2067–2073.

Falsanisi, D., Gehr, R., Liberti, L., Notarnicola, M., 2008. Effect of suspended particles on disinfection of a physiochemical municipal wastewater with peracetic acid. Water Qual. Res. J. Can. 43, 47–54.

Geeraerd, A.H., Valdramidis, V.P., Van Impe, J.F., 2005. GInaFit, a freeware tool to assess non-log-linear microbial survivor curves. Int. J. Food Microbiol. 102, 95–105.

Gehr, R., Wagner, M., Veerasubramanian, P., Payment, P., 2003. Disinfection efficiency of peracetic acid, UV and ozone after enhanced primary treatment of municipal wastewater. Water Res. 37, 4573–4586.

Harvey, M.S.; Howarth, J.N. Method of analyzing low levels of peroxyacetic acid in water. U.S. Patent 7,651–860, 2010.

Holt, C., Hirst, D., Sutherland, A., Macdonald, F., 1995. Discrimination of species in the genus listeria by fourier-transform infrared-spectroscopy and canonical variate analysis. Appl. Environ. Microbiol. 61, 377–378.

Jolivet-Gougeon, A., Sauvager, F., Bonnaure-Mallet, M., Colwell, R.R., Cormier, M., 2006. Virulence of viable but nonculturable S. typhimurium LT2 after peracetic acid treatment. Int. J. Food Microbiol. 112, 147–152.

Kitis, M., 2004. Disinfection of wastewater with peracetic acid: a review. Environ. Int. 30, 47–55.

Koivunen, J., Heinonen-Tanski, H., 2005. Peracetic acid (PAA) disinfection of primary, secondary, and tertiary treated municipal wastewaters. Water Res. 39, 4445–4453.

Lazarova, V., Janex, M.L., Fiksdal, L., Oberg, C., Barcina, I., Pommepuy, M., 1998. Advanced wastewater disinfection technologies: short and long term efficiency. Water Sci. Technol. 38, 109–117.

Lefevre, F., Audic, J.M., Ferrand, F., 1992. Peracetic acid disinfection of secondary effluents discharged off coastal seawater. Water Sci. Technol. 25, 155–164.

Liang, Y., Zhang, Z., Yang, X., Liu, W., 2013. Effect of suspended solids on the sequential disinfection of secondary effluent by UV irradiation and chlorination. J. Environ. Eng. 139, 1482–1487.

Lubello, C., Caretti, C., Gori, R., 2002. Comparison between PAA/UV and H_2O_2/UV disinfection for wastewater reuse. Water Supply 2, 205–212.

Luukkonen, T., Pehkonen, S.O., 2017. Peracids in water treatment: a critical review. Crit. Rev. Environ. Sci. Technol. 47, 1–39.

McDonnell, G., Russell, A.D., 1999. Antiseptics and disinfectants: activity, action, and resistance. Clin. Microbiol. Rev. 12, 147–179.

Monarca, S., Richardso, S.D., Feretti, D., Grottolo, M., Thruston, A.D., Zani, C., Navazio, G., Ragazzo, P., Zerbini, I., Alberti, A., 2002. Mutagenicity and disinfection by-products in surface drinking water disinfected with peracetic acid. Environ. Toxicol. Chem. 21, 309–318.

NRDC, 2011. Rooftops to Rivers II: Green strategies for controlling stormwater and combined sewer overflows. National Resources Defense Council, USA.

Oliver, J.D., Dagher, M., Linden, K., 2005. Induction of *Escherichia coli* and *Salmonella typhimurium* into the viable but not culturable state following chlorination of wastewater. J. Water Health 3, 249–257.

Rossi, S., Antonelli, M., Mezzanotte, V., Nurizzo, C., 2007. Peracetic acid disinfection: a feasible alternative to wastewater chlorination. Water Environ. Res. 79, 341–350.

Sanchez-Ruiz, C., Martinez-Royano, S., Tejero-Monzon, I., 1995. An evaluation of the efficiency and impact of raw wastewater disinfection with peracetic acid prior to ocean discharge. Water Sci. Technol. 32, 159–166.

Santoro, D., Bartrand, T.A., Greene, D.J., Farouk, B., Haas, C.N., Notarnicola, M., Liberti, L., 2005. Use of CFD for Wastewater disinfection process analysis: E. coli inactivation with peroxyacetic acid (PAA). Int. J. Chem. React. Eng. 3.

Stewart, P.S., Rayner, J., Roe, F., Rees, W.M., 2001. Biofilm penetration and disinfection efficacy of alkaline hypochlorite and chlorosulfamates. J. Appl. Microbiol. 91, 525–532.

USEPA, 1994. Combined Sewer Overflows (CSO) Control Policy. Washington, D.C., USA, United States Environmental Protection Agency.

USEPA, 1995. Combined Sewer Overflows Guidance for Long-term Control Plan. Washington, D.C., USA, United States Environmental Protection Agency.

USEPA, 1999. Combined Sewer Overflows Technology Fact Sheet Alternative Disinfection Methods. Washington DC, USA, United States Environmental Protection Agency.

Veschetti, E., Cutilli, D., Bonadonna, L., Brianesco, R., Martini, C., Cecchini, G., Anastasi, P., Ottaviani, M., 2003. Pilot-plant comparative study of peracetic acid and sodium hypochlorite wastewater disinfection. Water Res. 37, 78–94.

Wagner, M., Brumelis, D., Gehr, R., 2002. Disinfection of wastewater by hydrogen peroxide or peracetic acid: development of procedures for measurement of residual disinfectant and application to a physicochemically treated municipal effluent. Water Environ. Res. 74, 33–50.

Xue, Z., Hessler, C.M., Panmanee, W., Hassett, D.J., Seo, Y., 2012. Pseudomonas aeruginosa inactivation mechanism is affected by capsular extracellular polymeric substances reactivity with chlorine and monochloramine. FEMS Microbiol. Ecol. 83, 101–111.

Yong, H.N., Farnood, R.R., Cairns, W., Mao, T., 2009. Effect of sonication on UV disinfectability of primary effluents. Water Environ. Res. 81, 695–701.

Yuan, Z., Ni, Y., Van Heiningen, A.R.P., 1997. Kinetics of peracetic acid decomposition part I: spontaneous decomposition at typical pulp bleaching conditions. Can. J. Chem. Eng. 75, 37–41.