FOCUSED REVIEW

Kinetics and Mechanisms of Virus Inactivation by Chlorine Dioxide in Water Treatment: A Review

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Abstract
Chlorine dioxide (ClO₂), an alternative disinfectant to chlorine, has been widely applied in water and wastewater disinfection. This paper aims at presenting an overview of the inactivation kinetics and mechanisms of ClO₂ with viruses. The inactivation efficiencies vary greatly among different virus species. The inactivation rates for different serotypes within a family of viruses can differ by over 284%. Generally, to achieve a 4-log removal, the exposure doses, also being referred to as Ct values (multiplying the concentration of ClO₂ and contact time) vary in the range of 0.06–10 mg L⁻¹ min. Inactivation kinetics of viruses show two phases: an initial rapid inactivation phase followed by a tailing phase. Inactivation rates of viruses increase as pH or temperature increases, but show different trends with increasing concentrations of dissolved organic matter (DOM). Both damages in viral proteins and in the 5′ noncoding region within the genome contribute to virus inactivation upon ClO₂ disinfection.

Keywords Chlorine dioxide (ClO₂) · Virus · Disinfection · Kinetics · Mechanisms · Water treatment

Chlorine dioxide (ClO₂), an alternative disinfectant to chlorine, has been widely used to control a number of waterborne pathogens in water and wastewater treatment (AWWA Water Quality Division 2000; Sobsey 1989). Compared with chlorine, ClO₂ greatly reduces the generation of toxic halogenated disinfection products (Chang et al. 2000; Korn et al. 2002; Zhong et al. 2019), and chlorite and chlorate are the major ClO₂ byproducts (Gan et al. 2020; Schmidt et al. 2000; Sorlini et al. 2014). ClO₂ has a superior inactivation ability on bacteria such as Escherichia coli and Staphylococcus aureus (Huang et al. 1996), viruses such as poliovirus and adenovirus (Huang et al. 1997), fungi such as Penicillium chrysogenum and Stachybotrys chartarum (Wilson et al. 2005) and protists such as Cryptosporidium parvum (Chauret et al. 2001; Korich et al. 1990) and Giardia intestinalis (Winiecka-Krusnell and Linder 1998). Among these microorganisms, viruses consist of relatively simple structures and lack mechanisms to repair oxidative damage outside the hosts (Choe et al. 2015; Wigginton and Boehm 2020). However, viruses remain a concern as they exhibit higher resistance toward disinfectants than traditional bacterial indicators such as Escherichia coli and Enterococci and have very low infectious doses (Aronino et al. 2009; Fulton and Budd 1992; Mamane et al. 2007). The United States Environmental Protection Agency (USEPA) (2018) has included adenovirus, calciviruses, enterovirus, and hepatitis A virus in the contaminant candidate list 4 as common drinking water microbial contaminants. The World Health Organization guidelines (2011) for drinking water quality classify astroviruses, hepatitis E virus, sapovirus, and rotavirus as important pathogens with some evidence for high health risks. There are conclusive evidences that viruses (e.g. rotavirus, norovirus, enterovirus) can be disseminated through aquatic environments (IAWPRC Study Group on Water Virology 1983; Riera-Montes et al. 2011; Scarcella et al. 2009), though little is known about the fate of ongoing pandemic of COVID-19 in aquatic phase. Thus, to prevent the outbreak and epidemic of viruses, it is very important to ensure the effective inactivation of viruses during disinfection, a final barrier in the processes of drinking water or wastewater treatment.

At present, there are many studies on the virucidal activity of ClO₂ toward viruses, including nonenveloped viruses (e.g.
bacteriophage, enterovirus, adenovirus, calicivirus, rotavirus and parvovirus) and enveloped viruses (e.g. influenza virus, measles virus, herpesvirus and distemper virus). Enveloped viruses differ structurally from nonenveloped viruses due to the presence of a lipid bilayer membrane outside the viral protein capsid, which contains proteins or glycoproteins. The different functional groups on the outer surface of enveloped viruses compared to nonenveloped viruses likely impact their survival and partitioning behavior in aqueous environments (Arbely et al. 2006; Gundy et al. 2009; Shigematsu et al. 2014). Many factors have been found to exert great impacts on virus inactivation rates such as ClO2 dosage, pH, and temperature (Berman and Hoff 1984; Chen and Vaughn 1990; Hornstra et al. 2011; Thurston-Enriquez et al. 2005). The mechanisms of inactivation of virus by ClO2 include the disruption of the virus protein or the damage of genome (Jin et al. 2013, 2012; Li et al. 2004; Sigstam et al. 2013; Wigginton et al. 2012). Understanding the virus inactivation kinetics upon ClO2 disinfection is a pressing need in environmental engineering for ensuring sufficient disinfectant doses. By elucidating inactivation efficiencies and mechanisms of viruses, we can better control waterborne viruses in water and wastewater treatment.

As such, the purpose of this review is to provide an overview of the kinetics and mechanisms of inactivation of viruses by ClO2 disinfection, and identify the research gap and future research directions.

**Inactivation Efficiencies of Diverse Viruses**

The inactivation efficiencies of different kinds of viruses are shown in Table 1. Generally, the inactivation efficiencies of bacteriophage and rotavirus are very high and 4 log removal can be achieved within 0.06–1.45 mg L\(^{-1}\) min in disinfectant-demand-free water under different pH and temperature (Berman and Hoff 1984; Hauchman et al. 1986; Sanekata et al. 2010). Canine parvovirus is relatively difficult to be inactivated and no obvious inactivation was observed within 2 min at a ClO2 dosage of 1.0 mg L\(^{-1}\) (Sanekata et al. 2010). For the same kind of virus, cell-associated simian rotavirus SA11 is more resistant to ClO2 than freely suspended virions (Berman and Hoff 1984). Interestingly, for closely related viruses, they can exhibit very different susceptibilities to ClO2 than nonenveloped ones (Gallandat and Lantagne 2017; Rice et al. 2007; Ye et al. 2018). The explanation could be that the ClO2 can react with proteins on the enveloped membrane, such as the spike glycoprotein, the damage of which results in the failure of attachment to the host cell and thus the unsuccessful cell invasion and infection (Casais et al. 2003; Li et al. 2003; Yang et al. 2004). When the data of various viruses is put together (Fig. 1), it can be seen that viruses that are difficult to be inactivated by ClO2 are non-enveloped ones. Additionally, unlike UV disinfection, there is no obvious correlation between inactivation rates and genome types of viruses (e.g. single-stranded DNA, double-stranded DNA, single-stranded RNA and double-stranded RNA) during ClO2 disinfection. It may be because virus inactivation mechanisms by ClO2 differ between different viruses, which may be caused by genome damage or protein disruption.

In order to give a whole picture of the removal of diverse viruses in water, Fig. 1 shows the relationship between the log removal of viruses and Ct values. It should be noted that most viruses can be effectively removed within 4 mg L\(^{-1}\) min, however, some of enterovirus, calicivirus and adenovirus are difficult to be inactivated. According to the USEPA National Primary Drinking Water Standards (2003), public utilities must ensure a 4-log inactivation of viruses from source water. To meet this regulatory guideline, the Ct value must generally be more than 10 mg L\(^{-1}\) min. The threshold for chlorite in drinking water is set as 0.7 mg L\(^{-1}\) and 1 mg L\(^{-1}\) in China and USA, respectively (Ministry of
Table 1  Inactivation of viruses by chlorine dioxide (ClO2)

| Virus                  | Cₜ value (mg min L⁻¹)/t (min) | Inactivation | Experimental condition                  | Viral information                                      | References            |
|------------------------|-------------------------------|--------------|-----------------------------------------|--------------------------------------------------------|-----------------------|
| Bacteriophage          |                               |              |                                         |                                                        |                       |
| Bacteriophage f2       | 2 min  > 4 log                | 0.6 mg L⁻¹ ClO₂, pH 7.2, 5 °C | Nonenveloped virus with single-stranded RNA           | (Hauchman et al. 1986)                                  |                       |
| Bacteriophage f2       | 2 min  < 2 log                | 0.4 mg L⁻¹ ClO₂, pH 7.0, 5 °C | (Taylor and Butler 1982)                         | (Lim et al. 2010)                                       | (Hornstra et al. 2011) |
| Bacteriophage MS2      | 0.42 mg min L⁻¹ 4 log         | pH 7.2, 5 °C  | (Jin et al. 2013)                       | (Jin et al. 2013)                                       | (Taylor and Butler 1982) |
| Bacteriophage MS2      | 4 mg min L⁻¹ 5 log            | pH 7.2, 0 °C  | (Taylor and Butler 1982)               | (Hornstra et al. 2011)                                  | (Jin et al. 2013)     |
| Bacteriophage MS2      | 0.48 mg min L⁻¹ 4 log         | pH 7.2, 20 °C | (Jin et al. 2013)                       |                                                        |                       |
| Enterovirus            |                               |              |                                         |                                                        |                       |
| Enterovirus 71         | 3.93 mg min L⁻¹ 4 log         | pH 7.2, 20 °C | Nonenveloped virus with single-stranded RNA | (Jin et al. 2013)                                       |                       |
| Echovirus 11           | 1.0 mg min L⁻¹ 6 log          | pH 7.4       | (Zoni et al. 2007)                      | (Scarpino 1979)                                        |                       |
| Coxsackievirus A9      | 1.16 min 2 log                | 0.4 mg L⁻¹ ClO₂, pH 7.0, 15 °C | (Zoni et al. 2007)                          |                                                        |                       |
| Coxsackievirus B5      | 2.41 min 4 log                | 0.4 mg L⁻¹ ClO₂, pH 7.0, 20 °C | (Zoni et al. 2007)                          |                                                        |                       |
| Poliovirus             | 10 min 2 log                  | 1.0 mg L⁻¹ ClO₂, pH 7.0, 20 °C | (Alvarez and Brien 1982)                     | (Zoni et al. 2007)                                       |                       |
| Poliovirus             | 10 min < 2 log                | 0.4 mg L⁻¹ ClO₂, pH 7.0, 5 °C | (Taylor and Butler 1982)                    | (Zoni et al. 2007)                                       |                       |
| Hepatitis A virus      | 19.58 min 4 log               | 0.4 mg L⁻¹ ClO₂, pH 7.0, 20 °C | (Zoni et al. 2007)                          |                                                        |                       |
| Rotavirus              |                               |              |                                         |                                                        |                       |
| Human rotavirus        | 1.21 mg min L⁻¹ 4 log         | pH 7.2, 20 °C | Nonenveloped virus with double-stranded RNA | (Xue et al. 2013)                                       | (Chen and Vaughn 1990) |
| Human rotavirus type 2 | 1 min 4 log                   | 0.2 mg L⁻¹ ClO₂, pH 7, 5 °C | (Chen and Vaughn 1990)                     |                                                        |                       |
| Simian rotavirus SA11  | 0.37 min 4 log                | 0.17 mg L⁻¹ ClO₂, pH 7, 5 °C | (Chen and Vaughn 1990)                     |                                                        |                       |
| Simian rotavirus SA11  | 0.28 mg min L⁻¹ 2 log         | pH 6, 5 °C   | (Berman and Hoff 1984)                 |                                                        |                       |
| Cell-associated simian | 1.45 mg min L⁻¹ 4 log         | pH 6, 5 °C   | (Berman and Hoff 1984)                 |                                                        |                       |
| rotavirus SA11         |                               |              |                                         |                                                        |                       |
| Adenovirus             |                               |              |                                         |                                                        |                       |
| Adenovirus type 40     | 0.12 mg min L⁻¹ 4 log         | pH 8, 15 °C  | Nonenveloped virus with double-stranded DNA | (Thurston-Enriquez et al. 2005)                        | (Thurston-Enriquez et al. 2005) |
| Human adenovirus       | 2 min 1.5 log                 | 1.0 mg L⁻¹ ClO₂ | (Sanekata et al. 2010)                |                                                        | (Sanekata et al. 2010) |
| Canine adenovirus      | 2 min 0.5 log                 | 1.0 mg L⁻¹ ClO₂ | (Sanekata et al. 2010)                |                                                        |                       |
| Parovirus              |                               |              |                                         |                                                        |                       |
| Canine parvovirus      | 2 min 0 log                   | 1.0 mg L⁻¹ ClO₂ | (Sanekata et al. 2010)                |                                                        |                       |
| Calicivirus            |                               |              |                                         |                                                        |                       |
| Feline calicivirus     | 0.18 mg min L⁻¹ 4 log         | pH 8, 15 °C  | Nonenveloped virus with single-stranded DNA | (Thurston-Enriquez et al. 2005)                         | (Zoni et al. 2007)    |
| Feline calicivirus     | 9.59 min 4 log                | 0.4 mg L⁻¹ ClO₂, pH 7.0, 20 °C | (Sanekata et al. 2010)                  |                                                        | (Sanekata et al. 2010) |
| Feline calicivirus     | 2 min 0.25 log                | 1.0 mg L⁻¹ ClO₂ | (Sanekata et al. 2010)                |                                                        | (Sanekata et al. 2010) |
| Murine norovirus       | 0.25 mg min L⁻¹ 4 log         | pH 7.2, 5 °C  | (Sanekata et al. 2010)                |                                                        | (Lim et al. 2010)     |
| Influenza virus        |                               |              |                                         |                                                        |                       |
| Influenza A virus H1N1 | 5 min > 4.5 log               | 0.5 mg L⁻¹ ClO₂, 25 °C | Enveloped virus with single-stranded RNA | (Lénès et al. 2010)                                     | (Lénès et al. 2010)   |
| Influenza A virus H5N1 | 5 min > 4 log                 | 0.3 mg L⁻¹ ClO₂, 25 °C | (Lénès et al. 2010)                    | (Lénès et al. 2010)                                     |                       |
| Influenza virus        | 2 min 5 log                   | 1.0 mg L⁻¹ ClO₂ | (Sanekata et al. 2010)                |                                                        |                       |
Health 2006; USEPA 2006). In general, the applied ClO₂ dosage in drinking water disinfection is less than 1.5 mg L⁻¹ considering that 30–70% of ClO₂ is converted into chlorite (Schmidt et al. 2000; Sorlini et al. 2014; Yang et al. 2013). As such, the contact time for ClO₂ disinfection is generally in the range of tens of minutes.

**Effect of Experimental Conditions on Inactivation Kinetics**

Viruses inactivation by ClO₂ experiences an initial phase of pseudo first-order decay followed by a phase of slower kinetics or a tailing effect (Fig. S1). The occurrence of tailing is common in virus disinfection by ClO₂ including bacteriophage MS2 (Hornstra et al. 2011), enterovirus 71 (Jin et al. 2013), human and simian rotavirus (Berman and Hoff 1984; Chen and Vaughn 1990), murine norovirus (Lim et al. 2010), echovirus 11 (Zhong et al. 2017), enteric adenovirus and feline calicivirus (Thurston-Enriquez et al. 2005). It has been reported that heterogeneity of the virus population or virus attachment to other (virus) particles could be responsible for the tailing behavior (Gerba et al. 2003; Hornstra et al. 2011; Keswick et al. 1985; Thurston-Enriquez et al. 2003). However, Sigstam et al. (2014) suggested that the tailing behavior of MS2 was not caused by virus aggregation or by resistant subgroup, but due to the deposition of disinfection intermediates onto the virus capsid, protecting the viruses from further disinfection.

The initial fast inactivation phase of viruses exhibits a significant dose effect, that is, the inactivation rates increase with increasing ClO₂ dosages, but the second phase shows less difference (Fig. S1a) (Jin et al. 2013). Higher dose of ClO₂ has stronger disinfecting capacity (Katz et al. 1994), thus, it can inactivate viruses in a short time. However, when the contact time is longer enough, lower dose of ClO₂ can also effectively penetrate surface structure of viruses and lead to their death (Lin et al. 2014). Therefore, longer contact time may weaken the influence of dosage on the inactivation of viruses and remedy the insufficient disinfectant.

The virus inactivation rates in ClO₂ disinfection increase rapidly with increasing pH. For example, enterovirus 71 exhibits a higher inactivation efficiency toward ClO₂ at pH of 8.2 than pH of 5.6, as shown in Table 2 and Fig. S1b (Jin et al. 2013). Poliovirus is found to be inactivated 4.6 times faster at pH of 9.0 than that at pH of 7.0, and 8.3 times faster than that at pH of 4.5 at 21 °C (Scarpino 1979). The inactivation of bacteriophage f₂ increases by more than 5 log after treatment with ClO₂ for 2 min when pH increases from 5.0 to 9.0 (Taylor and Butler 1982). Enhanced inactivation with increasing pH is also observed in adenovirus type 40 (Thurston-Enriquez et al. 2005), feline calicivirus.

![Fig. 1](image_url) The log removal of viruses during ClO₂ disinfection. Note: the data of enveloped virus is circled; the line indicates the variations of Ct values to achieve the 4-log removal. The legend with a black edge indicates that Ct value is the product of initial concentration of ClO₂ and contact time.

**Table 1** (continued)

| Virus                         | Ct value (mg min L⁻¹)/t | Inactivation | Experimental condition | Viral information                                         | References            |
|-------------------------------|-------------------------|--------------|------------------------|----------------------------------------------------------|-----------------------|
| Other enveloped viruses       |                         |              |                        |                                                          |                       |
| Measles virus                 | 2 min                   | 1.75 log     | 1.0 mg L⁻¹ ClO₂        | Enveloped virus with single-stranded RNA                  | (Sanekata et al. 2010) |
| Human herpesvirus             | 2 min                   | 2.5 log      | 1.0 mg L⁻¹ ClO₂        | Enveloped virus with double-stranded DNA                  | (Sanekata et al. 2010) |
| Canine distemper virus        | 2 min                   | 3.75 log     | 1.0 mg L⁻¹ ClO₂        | Enveloped virus with single-stranded RNA                  | (Sanekata et al. 2010) |

*Ct values were used preferentially. For the papers without giving Ct values, time and initial ClO₂ concentration were provided, respectively. The classification of viruses is carried out according to the standard issued by International Committee on Taxonomy of Viruses (http://www.ictvonline.org/)*
Unlike dose effect, pH does not affect the initial inactivation phase so strongly, but seems to have an effect on the tailing phase. Noss and Olivieri (1985) hypothesized that the results may attribute to the change of the surface structure of the virion and the concentration of hydroxyl ion in the solution. In addition, individual virions in suspension may be induced to aggregate when the pH decreases due to the elimination of repulsive electrostatic force (isoelectric points of viruses are approximately 4.0). Viral aggregates have been reported to increase the survival of viruses in the environment and resistance to disinfectants (Clark 1968; Hoff and Akin 1986; Mattle et al. 2011). Therefore, lower pH is unfavorable for virus inactivation during ClO2 disinfection.

The inactivation rates of viruses by ClO2 are temperature-dependent and the inactivation efficiency is enhanced as temperature increases from 5 °C to 25 °C (Fig. S1c) (Jin et al. 2013). It is also reported that infectivity of enveloped bacteriophage Phi6 in droplets decreases by two orders of magnitude when the temperature increases from 19 °C to 25 °C (Prussin et al. 2018). It can be rationalized that the activation energy of ClO2 for killing viruses become lower at a higher temperature (Ji et al. 2008). However, in the study of Grunert et al. (2018), the inactivation rate constants of bacteriophage PRD1 slightly decreased when temperature increases from 15 °C to 25 °C, due to the decomposition of ClO2 at higher temperatures.

The inactivation efficiencies of viruses are also affected by water matrix. Studies have shown that better inactivation efficiencies of bacteriophage f2 and coxsackievirus B5 were observed in phosphate buffer solution than in hospital wastewater or municipal wastewater (Harakeh 1987; Taylor and Butler 1982; Wang et al. 2005; Zoni et al. 2007), primarily due to the competitive consumption of ClO2 by dissolved organic matter in wastewater or protective effect of particulates adsorbed on the viruses (Lin et al. 2014; Scarponi 1979; Fujioka et al. 1986). On the other hand, opposite trends are also observed. When the dissolved organic matter concentrations increased from 0.2 to 2.0 mgC L−1, bacteriophage PRD1 showed enhanced inactivation percentages and MS2 showed little difference in inactivation percentages upon ClO2 disinfection (Grunert et al. 2018). By-products, not chlorite and chlorate, were proposed to be responsible for the enhanced disinfection (Barbeau et al. 2005). Ammonia in water hardly affected the inactivation efficiency of bacteriophage f2 (Taylor and Butler 1982).

### Inactivation Mechanisms

For an infectious virus, it should be able to bind to its host cell, inject its genome inside the host cell, and replicate and translate once its genome gets into the host cell. All of these functions must be intact for the virus to be infective. In other words, to inactivate the virus, at least one of these functions must be destroyed.

Due to the different composition and three-dimensional structure of proteins and nucleic acids, the virucidal mechanism of ClO2 appears to be different for different types of viruses (Fig. 2). In bacteriophage such as MS2, fr and GA,
the mode of action of ClO2 mainly involves the degradation of the viral capsid proteins, which are largely responsible for interactions with the host cell and injection mechanisms (Hauchman et al. 1986; Noss et al. 1986; Sigstam et al. 2013; Wigginton et al. 2012). Therefore, the attachment of virus to host cells is inhibited, resulting in the inactivation of viruses. The denaturation of virus proteins is also reported to be the dominant inactivation mechanism upon ClO2 disinfection of human rotavirus and there is no genome damage (Xue et al. 2013). Zhu et al. (2019) suggested that destruction of membrane glycoprotein GP2a and GP4 by ClO2 blocked the interaction between porcine reproductive and respiratory syndrome virus (PRRSV) and cell receptors, leading to the termination of life cycle of this virus.

Considering the high reactivity of cysteine (1.0 × 10^7 M⁻¹ s⁻¹ at pH 7.0) (Ison et al. 2006), tyrosine (1.4 × 10^5 M⁻¹ s⁻¹ at pH 7.0) (Napolitano et al. 2005) and tryptophan (3.4 × 10^4 M⁻¹ s⁻¹ at pH 7.0) (Stewart et al. 2008) with ClO2 and their prevalence in diverse proteins, cysteine, tyrosine and tryptophan residues have been reported to be critical targets in the reaction between ClO2 and proteins, causing fragmentation and denaturation of proteins (Ogata 2007), though the reactivity of amino acid residue in protein are lower than that of free tryptophan (Ge et al. 2020). For example, ClO2 inactivation of influenza A virus is due to the oxidation of a tryptophan residue (W153) in the viral protein hemagglutinin, destroying its ability to bind with host cells (Ogata 2012). However, in enteroviruses such as poliovirus, enterovirus 71 and hepatitis A virus, ClO2 has been proposed to act on the viral genome. Specifically, the inactivation by ClO2 is caused by damage in the 5' noncoding region within the genome, which is necessary for the formation of new virus particles within the host cell (Jin et al. 2013, 2012; Li et al. 2004). Moreover, it has been reported that although protein damage plays an important role in inactivation of poliovirus, inactivation is ultimately attributed to viral RNA damage (Alvarez and O’Brien 1982; Simonet and Gantzer 2006). Disinfection resistance of viruses is closely related to these two kinds of inactivation mechanisms by ClO2 and the details are provided in Text S1.

**Future Perspectives**

This review summarized the inactivation efficiencies, kinetics and mechanisms of diverse viruses toward ClO2 based on the published literature. Further studies should focus on the causes of the tailing behavior, the effect of real water matrices on virus inactivation, and specific chemical modifications in the genome and capsid as well as their effects on viral structure and function (details in Text S2).

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