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On the cause of the tailing phenomenon during virus disinfection by chlorine dioxide

*Thérèse Sigstam, Andreas Rohatschek, Qingxia Zhong, Moritz Brennecke, Tamar Kohn**

Laboratory of Environmental Chemistry, School of Architecture, Civil and Environmental Engineering (ENAC), École Polytechnique Fédérale de Lausanne (EPFL), CH-1015 Lausanne, Switzerland

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ABSTRACT

This study investigates the mechanisms underlying the deviation from Chick–Watson kinetics, namely a tailing curve, during the disinfection of viruses by chlorine dioxide (ClO_2). Tailing has been previously reported, but is typically attributed to the decay in disinfectant concentration. Herein, it is shown that tailing occurs even at constant ClO_2 concentrations. Four working hypothesis to explain the cause of tailing were tested, specifically changes in the solution's disinfecting capacity, aggregation of viruses, resistant virus subpopulations, and changes in the virus properties during disinfection. In experiments using MS2 as a model virus, it was possible to rule out the solution's disinfecting capacity, virus aggregation and the resistant subpopulation as reasons for tailing. Instead, the cause for tailing is the deposition of an adduct onto the virus capsid over the course of the experiment, which protects the viruses. This adduct could easily be removed by washing, which restored the susceptibility of the viruses to ClO_2. This finding highlights an important shortcoming of ClO_2, namely its self-limiting effect on virus disinfection. It is important to take this effect into account in treatment applications to ensure that the water is sufficiently disinfected before human consumption.

1. Introduction

Chlorination is among the oldest and most commonly used disinfection process worldwide. However, over the years it has been shown that chlorine produces harmful by-products such as trihalomethanes and other halogenated compounds with potential carcinogenic effects (Xie, 2004). It is therefore of interest to investigate other disinfectants that have a similar disinfection potential but generate fewer problematic by-products. As a good alternative, chlorine dioxide (ClO_2) has shown to efficiently disinfect water for human consumption (Huang et al., 1997; Jin et al., 2013; Zoni et al., 2007). Importantly, it is effective at inactivating *Cryptosporidium*, whereas free chlorine is not (Chauret et al., 2001). Except from exhibiting a good disinfection capacity, ClO_2 can also oxidize iron and manganese, as well as help controlling taste and odor compounds (Aieta and Berg, 1986; Li et al., 1996). The disadvantage of using chlorine dioxide is that it reacts to chlorite, which may be neurotoxic at high doses (Xie, 2004).

In 1908, Chick published the first model for describing bacteria inactivation by disinfecting agents (Chick, 1908). The model suggests that the fraction of surviving organisms ($C_v/C_{v,0}$) exponentially decreases with time, which then leads to a linear decrease of ln ($C_v/C_{v,0}$) with time:

* *Corresponding author*. Tel.: +41 21 693 0891.
E-mail address: tamar.kohn@epfl.ch (T. Kohn).

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$$\ln(C_v/C_{v,0}) = -kt \quad (1)$$

Here, k represents the inactivation rate constant, C_v is the concentration of infective virus and t the time of disinfection. In order to be able to compare different disinfectant concentration, this model was expanded by Watson (1908) to yield the well-known Chick–Watson model:

$$\ln(C_v/C_{v,0}) = -k_{cw}C^n t \quad (2)$$

where k_{cw} is the Chick–Watson inactivation rate constant, C is the disinfectant concentration, and n is an empirical constant also called the dilution coefficient. Frequently it is found that $n = 1$, in which case the Chick–Watson model is first-order with respect to the disinfectant dose (expressed as Ct). This model thus allows calculating the disinfectant dose necessary to obtain a certain amount of inactivation. It was quickly discovered, however, that inactivation kinetics occasionally deviate from this simple model. In particular, inactivation curves frequently exhibit tailing after an initial exponential decay. The reason for this observed deviation divided the researchers into two main groups: the *vitalistics*, who argued that this deviation originated from heterogeneity in the population of microorganisms, and the *mechanistics*, who attributed these deviations to factors occurring during the disinfection process (Hiatt, 1964). To date, the mechanism underlying this deviation from Chick–Watson's first-order model still hasn't been fully assessed and understood (Harakeh and Butler, 1984). Cerf stated in his review on tailing of survival curves (Cerf, 1977) that: "People who have observed tails or who have considered the question, either accept tails as facts or reject them as artefacts". In other words, even though tailing is frequently observed, little attention has been given to its underlying cause. The occurrence of tailing, however, may lead to incomplete inactivation and ultimately may cause the disinfection process to fall short of the treatment goal. It is thus important to account for tailing, in order to ensure that water or food is sufficiently disinfected prior to human consumption.

Tailing appears to be particularly common in the case of virus disinfection by ClO_2. Examples include the inactivation of adenovirus, feline calicivirus, enterovirus 71, murine norovirus and human and simian rotavirus (Berman and Hoff, 1984; Chen and Vaughn, 1990; Jin et al., 2013; Lim et al., 2010; Thurston-Enriquez et al., 2005). Yet its occurrence was either not mentioned or simply attributed to the decay in chlorine dioxide concentration over time of reaction. In a recent study, Hornstra et al. (2010) performed an in-depth investigation on the disinfection of bacteriophage MS2 at low ClO_2 concentrations, and suggested that heterogeneity of the virus population (either in the original virus stock or acquired during disinfection) could be the reason for the tailing behavior. This hypothesis, however, was not proven, nor were other possible causes for the tailing behavior investigated in depth.

In the present work, we test the resistant subpopulation hypothesis, along with three other possible mechanisms that can lead to tailing: the presence of viral aggregates; changes in the solution properties during disinfection that diminish the efficiency of ClO_2; and changes in the virus properties during disinfection that protect them from ClO_2.

2. Materials and methods

Virus disinfection experiments were conducted in stirred dilution buffer (DB: 5 mM PO_4^{2-}, 10 mM NaCl, pH 7.4). MS2 was used as the test organism, because it is a commonly used surrogate for human viruses (Grabow, 2001) and to facilitate the comparison of our results with the study by Hornstra et al. (2010). At several time points during the inactivating treatment, samples were analyzed for the remaining virus infectivity. Experiments were typically conducted in two or more replicates with good reproducibility. Exceptions are the tests involving pretreatments with sonication, chloroform and filtration (see Section 3.2), which were conducted only once.

2.1. Chemicals

NaCl (99.5%), NaOH (extrapure), $NaH_2PO_4{\cdot}H_2O$ (99%), $K_2S_2O_8$ (99%), $NaHCO_3$ (99.7%) and $CHCl_3$ (99.8%) were purchased from Acros Organics (Geel, Belgium). $Na_2S_2O_3$ (98%), sinapinic acid (98%) and $NaClO_2$ (puriss.) was obtained from Sigma–Aldrich (Germany). HCl (25%) was obtained from Merck (Darmstadt, Germany). Ultrapure water (>18 $M\Omega cm^{-1}$) was used for all aqueous solutions.

2.2. Microorganisms

Bacteriophage MS2 (DSMZ 13767) and its *Escherichia coli* host (DSMZ 5695) were purchased from the German Collection of Microorganisms and Cell Cultures (Braunschweig, Germany). It was propagated as described previously (Pecson et al., 2009) and infectivity was assessed by enumeration of plaque forming units (pfu) using the double agar layer method (Walter, 1961).

2.3. Chlorine dioxide production and experimental setup

Chlorine dioxide was produced by mixing 100 mL 4% $K_2S_2O_8$ with 100 mL 2% $NaClO_2$ (Gates, 1998) and was stored at 4 °C. The resulting ClO_2 stock concentration (250–1000 mg/L) was determined by spectrophotometry (ε_{358nm} = 1200 $M^{-1}cm^{-1}$) (Hoigne and Bader, 1994). Prior to experiments, the stock solution was diluted to a working solution of 0.4–0.7 mg/L ClO_2, and was spiked with virus stock solution to a concentration of 0.5–1 × 10^{12} pfu/mL. To compensate for ClO_2 evaporation and consumption throughout the experiment, concentrated ClO_2 (16 mg/L) was added at a rate of 8–20 μL/min by means of a peristaltic pump (KdScientific). Prior to the start of each experiment it was ensured that this setup maintained a constant ClO_2 concentration under the given solution conditions. To halt the disinfection, ClO_2 was quenched by addition of sodium thiosulfate (0.63 M) at a 20:1 sample:quenching agent ratio. Control samples confirmed that the addition of sodium thiosulfate did not result in inactivation.

2.4. Re-growth of MS2 after inactivation

After disinfection, the solution was centrifuged using a 100 kDa Microcon centrifugal filter (Millipore, Billerica, MA)