Journal of Water Process Engineering 26 (2018) 46–54

Contents lists available at ScienceDirect

Journal of Water Process Engineering

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

Chlorine dioxide inactivation of *Pseudomonas aeruginosa* and *Staphylococcus aureus* in water: The kinetics and mechanism

Isaac Ofori[a], Suresh Maddila[a], Johnson Lin[b], Sreekantha B. Jonnalagadda[a,*]

[a] School of Chemistry & Physics, University of KwaZulu-Natal, Westville Campus, Chiltern Hills, Durban, 4000, South Africa
[b] School of Life Sciences, Discipline of Microbiology, University of KwaZulu-Natal, Chiltern Hills, Durban, 4000, South Africa

ARTICLE INFO

Keywords:
Chlorine dioxide
Pseudomonas aeruginosa
Staphylococcus aureus
Disinfection
Kinetics

ABSTRACT

This study evaluated the inactivation kinetics and the bactericidal mechanism of chlorine dioxide towards *Pseudomonas aeruginosa* (ATCC 27853) and *Staphylococcus aureus* (ATCC 29213) on a laboratory scale with the view of determining the optimal operational conditions of its application as an alternative disinfectant in water treatment. Bacteria inactivation was conducted in batch reactors at varied disinfectant concentrations, pH, temperature and initial bacteria densities in buffered disinfectant demand free water. The bactericidal mechanism in terms of the effect on the permeability of the outer and cytoplasmic cell membranes and the morphology of the cells were explored. At the highest studied concentration (5.0 mg/L), at least 5-log reductions in bacterial population were observed for each strain of bacteria. Chlorine dioxide inactivation showed a stronger sensitivity to changes in water pH conditions with the inactivation rate at 8.5 being at least 4-fold of what pertained at 6.5 but efficiency was less impacted by changes in the initial bacteria density. A rise in temperature from 4 °C to 15 °C resulted in approximately 56% increase in the inactivation rate of *S. aureus*. Chlorine dioxide was found to increase the permeability of outer and cytoplasmic cell membranes and consequently resulting in the release of vital nuclear materials which strongly correlated with loss of cell activity or death. However, from TEM micrographs significant morphological damages or cells lysis was not observed. These results provide vital data on operational strategies to enhance efficient disinfection of water with chlorine dioxide whilst monitoring regulatory requirements on disinfection by-products.

1. Introduction

Chlorine dioxide (ClO_2) is a powerful oxidant with broad-spectrum biocidal activity [1]. It has thus found considerable application as a sanitizer in the food industry [2,3], decreasing biofouling in industrial cooling water systems [4,5], disinfection of potable and wastewater [6,7] and in the pulp industry as a bleaching agent for producing excellent strong and bright fibers [1,8]. Most especially, in potable and wastewater disinfection, it has proven to be an excellent alternative technology to conventional chlorination against the threats of potential waterborne pathogenic and infectious agents such as bacteria, viruses, and protozoa [9–11]. Besides the regular application as a disinfectant in water treatment, recent reports have also indicated promising potentials for the oxidation and removal of pharmaceutical residues such as diclofenac [12], tetracyclines [13] and sulfamethoxazole [14] in wastewaters.

Chlorine dioxide inactivates microbial threats by selective oxidation where a single electron is usually abstracted. This results in the formation of chlorite (ClO_2^-) and chlorate (ClO_3^-) as the main by-products with chlorite constituting approximately 70% [15] of the total reaction by-products.

$$ClO_2 + e^- \rightarrow ClO_2^- \quad (1)$$

$$2ClO_2 + 2OH^- \rightarrow ClO_2^- + ClO_3^- + H_2O \quad (2)$$

Chlorites levels in drinking water are increasingly being regulated due to potential health concerns associated with them. The WHO recently reaffirmed a maximum acceptable threshold for chlorite in drinking water as 0.7 mg/L [16]. The US EPA also regulates the maximum contaminant level (MCL) for chlorite in drinking water at 1.0 mg/L [17]. Consequently, the maximum residual disinfectant levels (MRDL) for chlorine dioxide in drinking water is enforced at 0.8 mg/L in the United States. The associated challenge in the potable water treatment industry thus lies with satisfying the regulatory guidelines without

* Corresponding author at: School of Chemistry & Physics, University of KwaZulu-Natal, Durban, 4000, South Africa.
E-mail addresses: ofass2006@yahoo.co.uk (I. Ofori), sureshmskt@gmail.com (S. Maddila), linj@ukzn.ac.za (J. Lin), jonnalagaddas@ukzn.ac.za (S.B. Jonnalagadda).

https://doi.org/10.1016/j.jwpe.2018.09.001
Received 18 April 2018; Received in revised form 6 August 2018; Accepted 1 September 2018

compromising the adequacy of disinfection. This is further exacerbated by the increasing reports of some bacterial strains developing resistance to antibiotics in water due to inadequate disinfection [18,19].

In this regard, designing and operating an efficient chlorine dioxide disinfection system for either potable water or wastewater and simultaneously controlling the formation of deleterious disinfection by-products would require due consideration of suitable operational conditions to a target microorganism. In a recent communication [20], we assessed the efficiency of chlorine dioxide as a disinfectant against *Escherichia coli* under different operational conditions of water treatment in addition to a proposed bactericidal mechanism. However, it is well established that the intrinsic susceptibilities of organisms to disinfectants vary widely [21,22] and thus an appropriate determination of optimal operational conditions of a disinfectant would partly be dependent on the target organism. A broader data regarding the disinfection of other bacterial species with chlorine dioxide thus becomes necessary.

Pseudomonas aeruginosa is a Gram-negative bacterium that is ubiquitous in the environment and able to adapt to several conditions. It could also be found in water including swimming pools, hot tubs and tap water [23–25]. Indeed the transmission via hospital tap water has been described as highly significant [26]. There are even reports of *P. aeruginosa* having been isolated from bottled water [25]. It is often described as an opportunistic pathogen due to its association with life-threatening ailments in burn and surgical patients and in immunocompromised patients in hospital environments. It is also a common cause of infections in patients suffering from cystic fibrosis [27]. *Staphylococcus aureus*, on the other hand is prevalent in diverse food products such as meat and milk [28,29]. Additionally, it could as well be found in environmental water samples [30,31]. The methicillin-resistant *S. aureus* (MRSA) strains have particularly gained considerable attention in recent times owing to the risks of transmitting potential infections from animals to humans [32]. In previous related studies designed to monitor the efficiency of chlorine dioxide at controlling these strains of bacteria, relatively high concentrations of the disinfectant were employed [33,34]. Such concentrations however, may not be suitable for satisfying regulatory requirements when applied for disinfecting drinking water. Moreover, the available data from a systematic study in literature involving the use of chlorine dioxide to inactivate *P. aeruginosa* and *S. aureus* is quite limited

In the present work, the kinetics of chlorine dioxide inactivation of *P. aeruginosa* and *S. aureus* were studied under varied conditions of oxidant concentration, water pH, temperature and initial bacterial density. The bactericidal mechanism of chlorine dioxide on the bacteria strains was also investigated.

2. Materials and methods

2.1. Bacterial strains and preparation of suspension

Culture collection strains of *P. aeruginosa* (ATCC 27853) and *S. aureus* (ATCC 29213) were obtained from the Microbiology Discipline, University of KwaZulu-Natal, Westville. Bacterial cell suspensions were prepared for the inactivation studies by growing the stock cultures in 100 mL of Lysogeny broth (LB) (Merck, South Africa) contained in a 250 mL Erlenmeyer flask and incubated for 18–24 h at 37 °C. Cells were then harvested by centrifuging at 6000 *x g* for 5 min at 4 °C, washed twice in phosphate buffered saline (PBS, 10 mM at pH 7.4) and pellets suspended in an oxidant demand free (ODF) buffered water to an initial density of approximately 10^8 cfu/mL.

2.2. Chlorine dioxide preparation and measurement

Stock solutions of chlorine dioxide were prepared as described previously [20,35] by oxidizing approximately 25% (w/v) solutions of sodium chlorite ($NaClO_2$) in a gas generating bottle with a dilute solution of sulphuric acid (H_2SO_4, 2 M). The generated gas was harvested through a stream of compressed air into a connecting chlorine scrubber system which contained a saturated solution of sodium chlorite (10% w/v) to scrub contaminants such as chlorine gas. Chlorine dioxide gas was collected in a connecting bottle of demand free deionized water. The concentrations of the prepared stock solutions were analyzed by the Iodometric method whilst the residual concentrations were determined by the *N*, *N*-diethyl-p-phenylenediamine (DPD) method [35].

2.3. Bacteria inactivation kinetics

Each of the inactivation kinetic reactions of *P. aeruginosa* and *S. aureus* with chlorine dioxide were carried out in a 500 mL of sterile oxidant demand free (ODF) deionized water buffered with phosphate buffered saline (PBS) in a glass batch reactor. Prior to each inactivation reactions, glasswares were soaked in 10 mg/L chlorine dioxide solution for 4 h and thoroughly rinsed with deionized water to remove all possible chlorine dioxide consuming species that might interfere with the applied dose during the reaction. Bacterial suspensions estimated to yield a final concentration of ~ 10^8 cfu/mL based on prior calibrations were added to the water in the reactor and allowed to equilibrate with the surrounding temperature under continuous stirring with a magnetic stirrer to ensure uniform mixing throughout the reaction period. Samples of bacterial suspensions from the reactor were withdrawn before the addition of the chlorine dioxide to determine the initial bacteria populations. Subsequently, calculated desired concentrations of chlorine dioxide (0.5, 1.0, 2.5, 3.5 and 5.0 mg/L) were added to initiate the reaction in the reactor. Five milliliters samples were withdrawn with 10 mL sterile syringes at predetermined time points into tubes containing excess 0.1 M sodium thiosulphate ($Na_2S_2O_3$) solutions to immediately quench the residual chlorine dioxide and consequently stopping the reaction. Enumerations of bacteria were determined by the standard spread plate technique on nutrient agar (Merck, South Africa) after appropriate dilutions and incubated at 37 °C for 24 h.

The influence of water temperature and pH on the rate and degree of chlorine dioxide inactivation of each bacteria strain considered in this study were also assessed. Inactivation kinetic reactions as described above were conducted at different temperatures with the reaction water matrix at pH 8.05 maintained at 4, 15, 22, 30 and 37 ± 2 °C and allowed to equilibrate with the surrounding temperature before initiating the reaction. Chlorine dioxide was applied at an initial dose of 2.0 mg/L in each case based on preliminary trials at room temperature which yielded at least 2-log (99%) inactivation credits of each bacteria. Such levels of reduction were considered appropriate to indicate possible significant effects of the operational parameters on inactivation. To determine the effect of changes in water pH on the inactivation kinetics, experiments were similarly carried out at 22 ± 2 °C by varying the pH in the range of 6.5–9 in relation to the pH conditions usually encountered in natural water. It should be noted that all inactivation experiments for each bacterium were carried out independently and measurements made in triplicates.

2.4. Effect of bacterial density on the kinetics of ClO_2 disinfection

The bacteria cultures of the studied organisms were grown for 18–24 h into the stationary phase in a Lysogeny broth under agitation at 37 °C. Cells were then harvested by centrifugation at 6000 x *g*, washed twice with sterile PBS (PH 8.05) and subsequently resuspended in a buffered 100 mL ODF water to obtain the stock bacteria density at an approximate count of 1–2 × 10^8 cfu/mL. This was estimated from a predetermined data between optical densities (OD) measured at 600 nm with a spectrophotometer and cell density (cfu/mL). The stock cultures were serially diluted to obtain approximate cell densities of 10^7, 10^6 and 10^5 cfu/mL in 100 mL of the reaction matrix contained in 250 mL Erlenmeyer flasks. Each of the different densities of *S. aureus* and *P.*