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Disinfection of water in a batch reactor using chloridized silver surfaces

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ABSTRACT

There is growing interest in the use and development of silver based disinfection technologies owing to its safe and effective bactericidal action. The present work demonstrates the bactericidal effect of chloridized silver wires, where chloridization was achieved through chemical/electrochemical methods. The disinfection kinetics of uncoated and chloridized silver wires immersed in a 100 mL batch reactor were compared for one Gram positive and two Gram negative model bacterial strains, *i.e.*, *Bacillus subtilis* MTCC 441 and *Escherichia coli* MTCC 443 and MTCC 739, respectively. Results showed that the chloridized silver wires could achieve better bactericidal effect compared to the uncoated silver wires. Initial cell concentration (N_0) had a significant influence on the time required for complete disinfection which increased by more than 60 fold as N_0 increased from 10^3 to 10^9 CFU/mL. The disinfection kinetics was correlated with release of silver ions from the chloridized surfaces and was affected by changes in water quality and presence of other constituents in water. High alkalinity showed minimal adverse effect whereas high values of hardness and natural organic matter had severe adverse effect on disinfection kinetics and silver release.

1. Introduction

Pathogenic microorganisms in water cause waterborne diseases such as diarrhea, dysentery, cholera and typhoid. Although chlorine has been used as a disinfectant for decades there are serious concerns over disinfection by-products (DBPs) formed when chlorine reacts with natural organic matter [1,2]. These DBPs, are potentially hazardous and may result in spontaneous abortion, cardiovascular defects, neural tube defects and even cancer [3,4]. This has revived interest in use of silver for disinfection. Silver compounds have been developed and used as water disinfectant and antimicrobial coating on surgical materials for many decades [5]. Silver salts are widely reported to be effective in inactivating a broad spectrum of microorganisms and they do not impart taste, odor and color to water. Silver manifests antibacterial activity in all the forms, *i.e.*, ionic form, metallic form and in the form of nanoparticles [6]. The most widely known bactericidal mechanism of silver ion is its interaction with the thiol group of L-cysteine residue of proteins and inactivation of their enzymatic functions [7]. Other suggested mechanisms for silver disinfection include inhibition of DNA replication [8], release of intracellular potassium [9], generation of intracellular reactive oxygen species [7], damage to cell wall and detachment of cytoplasmic membrane from cell wall [8]. Recent studies have suggested that the effectiveness and broad spectrum activity of silver lies in its ability to attack multiple targets sites in the bacterial machinery and thus it becomes difficult for bacteria to develop resistance against silver.

Researchers have demonstrated the antibacterial activity of silver nanoparticles and nanocomposites due to continuous release of silver ions into water and also due to direct action of nanoparticles on bacterial membranes [10–13]. The synergism and enhancement in disinfection ability of silver when used in combination with Al_2O_3 [14,15], H_2O_2 [16], and UV radiation [17] has been well documented. Immobilization of silver nanoparticles on thin alumina substrates [18], zinc oxide [19], zeolite [20,21], and TiO_2

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[22] and various other materials [11,23–25] have been reported for development of antimicrobial surfaces for water disinfection. Silver nanoparticles on AgCl and BiOCl are reported to have enhanced photocatalytic activity upon visible light irradiation [26,27], however, disinfection using such surfaces have not been explored. There are only limited reports on use of AgCl/AgCl coating on silver for water disinfection [28].

With traditional disinfectants water quality has been found to be of prime importance in effectiveness of disinfection and also in generation of disinfection by-products. Disinfection with chlorine and UV irradiation is ineffective in the presence of natural organic matter (NOM, humic and fulvic acids) and other particulates [29,30]. The nature and concentration of NOM affects the formation of DBPs [31] and NOM is also reported to coat the surfaces of bacteria to protect them from the action of conventional disinfectants, such as UV and chlorine [32]. Within the Indian subcontinent, ground water, ponds and lakes serve as the sources of drinking water. The quality of these water resources depends on various water quality parameters which influence its beneficial use for humans and also determine the sustainability of ecosystems. Moreover, water sources from different geographical regions show significant variation in water quality. A few recent studies have reported that the effectiveness of silver based disinfection can be affected by water quality [33,34]. Therefore, it is of importance to evaluate disinfection performance of silver for varying water quality and determine its efficacy under practically relevant conditions.

In the context of disinfection using silver, water quality will not only affect the total aqueous concentration of silver, it is also expected to affect silver speciation. In studies with silver nanoparticles various constituents in water and biological media are reported to affect the bactericidal effect due to changes in the release of silver ions and due to formation of complexes with ligands in solution [35,36]. NOM present in water is reported to reduce the bactericidal effect due to silver binding by humic acids [37,38]. Organic S and N-ligands may also bind silver through complexation reactions and alter its bioavailability. In media containing chloride, silver is reported to form complexes with chloride ions, such as, $AgCl_2^-$ and $AgCl_3^{2-}$ [39]. The bactericidal effect of various inorganic and organic complexes of silver has not been studied extensively.

The objective of this study was to explore the feasibility, extent and kinetics of disinfecting water using chloridized silver surfaces, in systems devoid of nanoparticles/nanofibers. The relative ease of regenerating a surface coat of AgCl either chemically or electrochemically for repeated use was the motivation for this study. It was hypothesized that the surface coat of AgCl would affect the rate of release of silver ions and the rate of disinfection. In India, it is a common practice to store water in containers before consumption although contamination and proliferation of microbes during storage has been reported. With an ultimate objective of developing a device that would be useful under such conditions, all disinfection studies were designed under static conditions with no power input for mixing. Another objective was to explore the impact of various factors, *i.e.*, type of bacteria, initial concentration of bacteria and impact of water quality, on disinfection kinetics and the time required for complete disinfection in presence of chloridized silver wires. Since water chemistry potentially affect the toxicity, bioavailability, and fate of silver in a system, understanding the impact of water chemistry on silver release and disinfection effectiveness was considered to be of practical relevance in application of such a silver based disinfection devices. No prior studies have explored the effect of such chloridized surfaces for disinfection and compared its performance to that of silver surfaces under various water quality conditions for multiple bacterial strains present at various concentrations.

2. Materials & methods

2.1. Bacterial cultures and culture preparation for disinfection studies

The experiments were carried out using the gram negative bacterial strains, *Escherichia coli* MTCC 443, *E. coli* MTCC 739 and a gram positive strain *Bacillus subtilis* MTCC 441 procured from Institute of Microbial Technology (IMTECH, Chandigarh, India). The bacterial cultures were plated on chloride free Eosine-Methylene blue (EMB) agar medium (Himedia, Mumbai, India) having the following composition: peptic digest of animal tissue (10 g/L), di-potassium phosphate (2 g/L), lactose (5 g/L), eosin-Y (0.40 g/L), methylene blue (0.065 g/L) and agar (13.50 g/L). Each bacterial strain was grown in nutrient broth by incubation in a rotary shaker (30 °C, 180 rpm) up to end of log phase (24–28 h), harvested and washed twice in phosphate buffer by centrifugation at 10,000 rpm at 4 °C for 10 min. The nutrient broth contained peptic digest of animal tissue (5.0 g/L), NaCl (5 g/L) and beef extract (1.5 g/L). Initially disinfection was tested directly in nutrient broth after growing the cells up to end of log growth phase. Subsequently, the harvested and washed cells were suspended in deionized (DI) water so as to obtain absorbance of unity at 600 nm. Thereafter, samples having the desired initial cell concentration ($\sim 10^3$, 10^4, 10^5 and 10^9 CFU/mL) were prepared by appropriate dilution. For the various initial counts up to 10^5 CFU/mL, time for complete disinfection was determined. When the viable counts were reduced to zero, an aliquot withdrawn from the reactor was added to nutrient broth and incubated in a rotary shaker to confirm the absence of viable cultures. Possible conversion of cells to the viable and not culturable state (VBNC) was also verified by staining with the Baclight kit (Molecular Probes Inc., USA) and observing in a fluorescence microscope (Axio Imager A2, Carl Zeiss, Germany) using Fluorescein isothiocyanate (FITC) and Rhodamine B filters before and after completing disinfection for all the three strains exposed to chloridized silver surfaces at an initial count of 10^3 CFU/mL.

2.2. Disinfection studies with natural water samples

Natural water samples from Powai Lake (Mumbai, India) and from a pond in its vicinity (IIT Bombay, Mumbai, India) were collected. Water quality parameters such as pH, alkalinity, chlorine content, hardness and chemical oxygen demand (COD) were analyzed as specified in Standard Methods [40]. Initial viable count in each sample was enumerated by serial dilution and subsequent plating on nutrient agar/EMB agar before initiating the disinfection studies with these natural water samples.

2.3. Disinfection studies with simulated lake water

A synthetic lake water was prepared following Butkus et al. [17], *i.e.*, dextrose (30 mg), $NaNO_3$ (6.5 mg), Na_2SO_4 (22.5 mg), NH_4Cl (33.3 mg) and $NaHCO_3$ (25.2 mg). The model lake water composition, taken as "reference" in the following studies was formulated with 31.6 mg/L alkalinity, and zero hardness and humic acid. Using this as a 'reference', water with varying alkalinity was prepared by adding appropriate quantities of Na_2CO_3 and $NaHCO_3$. Hardness of the water was adjusted using $CaCl_2$ and $MgCl_2$. Humic acid was taken as the surrogate for background organic matter. For synthetic lake water with varying alkalinity, hardness and organic matter, disinfection studies were performed with *E. coli* MTCC 443 and 739 and *B. subtilis* MTCC 441 for initial cell count of 10^3 CFU/mL.