Research Article
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Effects of storage conditions on superoxidised water

Abstract

Superoxidised water (SOW) in the food industry has several advantages over traditional chemical disinfectants. SOW is non-toxic and environmentally friendly and does not leave any harmful by-products. In addition, SOW is easy to use and can be applied to various food surfaces. SOW has been used to avoid the food industry's biological pollution caused by food-borne bacteria pathogens with recognized as a short producing time and evident in the sterilization process, SOW is highly recommended for its eco-friendly ability. Chlorine-containing solutions such as SOW are used for broad disinfection purposes, including in the food industry. Still, their effectiveness and stabilities often need to be investigated as residues such as chlorine in SOW are not stable. The efficacy of SOW can be easily affected by several factors, including pH, temperature, and organic matter content. In this study, the two different storage conditions influenced by the light exploration were used to investigate the light condition that directly impacts the chlorine level in SOW. Light intensity plays an important role in determining chlorine loss from SOW. Chlorine dissipation rates in the light condition are higher than in the dark condition. With the initial 40+0.00 mg/L of chlorine, the chlorine level was decreased as plastic bottle/light > glass bottle/light > plastic bottle/without light> glass bottle/without light. They show that the change in chlorine level is significantly influenced by the type of bottle and the environmental condition, such as light intensity, in this study. The results obtained in this study validate the practice of SOW's storage condition to slow down chlorine's bulk decay when applied in various industries, such as the food industry.

Keywords: superdioxised water, disinfectant, chlorine, storage condition, light exposure

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Introduction

Due to the potential for the emergence of more than 300 infectious diseases with a capability not previously known to human beings, contagious diseases are a significant challenge for humans.[1] Despite the recent adaptation of hygiene to biomedical (hospitals), education (schools/colleges), the surrounding environment (air/water), and industry (food/textile/animal husbandry), it is an increasingly critical public health issue worldwide. Various substances have been introduced into the public domain to increase sanitation among humanity. A disinfectant is one of these products. Various disinfectants on the market can kill microbial pathogens or inhibit their growth, including some microorganisms.[2] A disinfectant can be categorized into three levels: a low level, a middle level (medium level) or an upper level.[3] A low level of disinfection should only be applied to unmedical devices and environmental areas that will come in contact with the skin. It is recommended to disinfect uncritical devices which come into contact with intact skin to an intermediate level of disinfection. One example of this disinfectant is superoxidised water. Superoxidised water is a novel antiseptic solution sold worldwide in pharmacies.[4] It has been stated that this solution is produced by exposing sodium chloride through a semi-permeable membrane and then using electrolysis to produce oxychloride ions.[5] This solution works by attacking the walls of free-range microbes without harming the ions within human cells.[6] It has been used in many cases of ulcers and diabetic feet. It is generated by exerting an electrical current on salty water and causing an electrochemical reaction in aqueous solutions from water and sodium chloride.[7] Water is reduced to oxygen, ozone, and other reactive species; however, the primary ingredient formed during this process is hypochlorite and hypochlorous.[8] In the past 25 years, super-oxidized solutions have been potent antimicrobial agents and disinfectants through oxidative damage.[9] Electrolyzed water contains a mixture of inorganic oxidants, such as hypochlorous acid (HClO), hypochlorous acidic ion (ClO^-), chlorine (Cl_2), hydroxide (OH), and ozone (O_3), which are effectively inactivating a variety of microorganisms responsible for endodontic infections.[10]

Chlorine is one of the many components that affect the disinfectant ability of superoxidised water. This is because when the water is mixed with chlorine compound, it forms a reaction called chlorination.[11] Chlorination is the most common method for disinfecting secondary treated wastewater before final disposal in rivers, lakes, or oceans. The predominant use of chlorine in disinfection is a well-known technique with a broad spectrum of germicidal activity and a low-cost.[12]

Chlorine is a volatile compound that can significantly impact the properties of superoxidised water. Understanding the factors that influence the concentration and behaviour of chlorine in superoxidised water is crucial for optimising its use in various applications. Chlorine-containing disinfectants were widely used during the COVID-19 pandemic. [13] Sattar et al.[14] reported that chlorine-based disinfectants are more effective in reducing bacterial contamination on stainless steel surfaces in hospital settings than alcohol-based disinfectants. This study suggested that the superior disinfectant properties of chlorine-based products may be due to their ability to penetrate biofilm, which can form on stainless steel surfaces and provide a protective barrier against disinfectants.

Oliveira et al.[15] found that chlorine dioxide was more effective than alcohol-based disinfectants in reducing bacterial contamination on stainless steel surfaces in food processing. This concluded that chlorine dioxide could be an effective alternative to alcohol-based disinfectants in the food industry. Macedo et al.[16] investigated the effectiveness of various disinfectants on stainless steel surfaces in a hospital setting. In this study, researchers found that chlorine-based disinfectants were more effective in reducing bacterial contamination on stainless steel surfaces than alcohol-based disinfectants. The researchers suggested that this could be due to biofilm on the surfaces.

In recent studies, many variables could influence the stability of superoxidised water. This experiment aims to determine the two factors that influence the strength of superoxidised water. The variables experimented on are light intensity and type of superoxidised water container. The parameter used in this experiment is the chorine content in the superoxidised water.

Material and methods

Superoxidised water preparation

The superoxidised water was prepared using an SOW-generated machine at UCSI University, Malaysia. It has an oxidation-reduction potential of about 960mV and an active free chlorine concentration of 650-750 ppm. The pH is 7.05, which can meet the endoscope disinfection standard.

Incubation preparation

Clear glass bottles (250 mL) and clear plastic bottles (250 mL) were prepared to measure the chlorine level after exposure to sunlight and kept in a dark condition. Bottles were fulfilled (200 mL SOW) and stored in the selected condition for four weeks. The storage temperature was 30+2°C for dark conditions and 32+2°C for sunlight conditions. The chlorine test was done once weekly to observe the chlorine concentration after 1 week of incubation in mg/L. Triplicate reading is taken during chlorine testing to ensure the data is accurate.

Analytical test

Chlorine testing was done to measure the chlorine concentration of the superoxidised water solution. The solution is taken from the incubation places. 10 ml of the sample was transferred into a beaker glass and then diluted with distilled water. This is to lower the chlorine concentration so the colour indicator in the chlorine test kit can detect it. Two drops of chlorine reagent were used. This is to measure the chlorine concentration inside the solution. The solution then shakes and is put in the tube rack for 10 minutes. This ensures that the chlorine concentration inside the solution is accurately detected. The colour indicator is used to detect the chlorine concentration. The experiment was repeated three times to get the triplicate reading from the sample. The original samples were put back into the incubation place after testing. The kinetics of chlorine reaction in SOW are using the formula below:

$$C = C_o e^{-K_b t}$$

Where: C is the chlorine concentration at the time t; Co is the chlorine concentration at time zero; t is the time; K_b is the constant reaction rate in week^{-1}.

Data analysis

Statistical analysis was done in Statistical Package for the Social Sciences (SPSS). The data were analysed via ANOVA followed by the Turkey test to assess the significance of chlorine loss among the tested groups.

Results and discussion

Superoxidised water, also known as electrolysed water, has been widely used as an alternative in asepsis and sanitisation worldwide. Super-oxidised waters have been researched as disinfectants for instruments and hard inanimate surfaces in hospitals. In endoscope disinfection, for example, SOWs have decreased the time, toxicity, and costs of material disinfection.[17]

Microbial control due to food delivery and packaging management is one of the consent in the food safety industry. With the United Nations Sustainable Development Goals (UN-SDGs)- SDG Goal 2: Zero Hunger to achieve food security and improved nutritional value of food, and SDG Goal 12: Sustainable consumption and production, industries and stakeholders and encouraged to create more sustainable food products by reducing the food waste. With this study, the researchers aim to investigate light intensity's influence on the SOW's chlorine level. With its antimicrobe mechanism, SOW was applied in the food industry to prolong the shelf life. Gupta et al.[18] show that the growth of pathogen bacteria such as *Staphylococcus aureus* and *Escherichia coli* can be inhibited by SOW. With the initial 40+0.00 mg/L of chlorine, the chlorine level was decreased as plastic bottle/ light > glass bottle/light > plastic bottle/without light> glass bottle/ without light (Table 1).

Table 1 Chlorine level after 4 weeks of treatment, with different light intensity and type of container

Weeks	Chlorine Level (mg/L)			
	With light		Without light	
	Glass bottle	Plastic bottle	Glass bottle	Plastic bottle
0	40.00 ± 0.00^{A}	40.00 ± 0.00^{a}	40.00 ± 0.00^{AA}	40.00 ± 0.00^{aa}
1	26.66 ± 0.06^{B}	16.66 ± 0.09^{b}	36.66 ± 0.00^{BB}	28.33 ± 0.03^{bb}
2	16.66 ± 0.06^{C}	8.33 ± 0.09^{C}	25.00 ± 0.00^{CC}	20.00 ± 0.00^{cc}
3	11.00 ± 0.43^{D}	6.66 ± 0.05^{d}	20.00 ± 0.03^{DD}	16.33 ± 0.05^{dd}
4	8.00 ± 0.00^{E}	5.00 ± 0.30^{e}	15.00 ± 0.00^{EE}	13.33 ± 0.00^{ee}

Note: Data are expressed as means±standard deviation value (n=3). The superscript letters within the column differ significantly according to the Turkey test ($p<0.05$) among the mean value. Different lower-case letters indicate a significant difference among all types of treatments.

It is stated that exposure to sunlight can significantly enhance the degradation of superoxidised water, leading to the formation of various reactive oxygen species. Therefore, storing super oxidized water in a cool, dark place is important to maintain its stability and potency. Sugiyama et al.[19] as the degradation rate of chlorine was strongly dependent on the intensity of sunlight exposure, with higher intensity leading to more rapid degradation.[20] As reported by García-Ávila et al.[21] chlorine in water shows a decomposition rate of 0.15 h^{-1}, and it shows that temperature is one of the parameters that affect the variation of the reaction rate of chlorine in a water source.

The concentration of hypochlorous acid (HOCl), a key active ingredient in superoxidised water, decreased significantly in both containers over time.[22] The stability of HOCl in a solution is influenced by the storage condition and mechanism of storage.[23] The researchers attributed this to the permeability of plastic to oxygen, which could lead to the oxidation of HOCl.[24] HOCl decomposes at room temperature at a low rate and significantly increases when temperature. In addition, the movement of the bottles and the amount of solution in a bottle also enhance the decomposition of HOCl in the solution. This is due to the vibration or movement of the solution impacting the water's contact with the air inside the containers. In this study, bottles involved in the experiment were only filled with 80% of the SOW for all treatments. This might significantly affect the readings.

Tixier et al.[25] evaluated the effect of storage temperature and container type on the stability of superoxidised water over 28 days. The results showed that superoxidised water stored in glass bottles had a higher retention of HOCl concentration than those stored in plastic bottles. This is because the hypochlorous acid's high retention, the superoxidised water's disinfectant agent, can be retained more when the solution is stored in a glass container than in a plastic container The researchers attributed this to the ability of glass to protect the solution from environmental factors such as light, heat, and oxygen.[25]

Table 2 shows the comparison of all variables that have been tested that could influence the stability of chlorine content of superoxidised water. As seen in Table 2, the significant value of the data is 0.03 this is smaller than the expected significant value (P>0.05). Therefore, the null hypothesis was rejected. This means that there are significant differences in the chlorine concentration between each variable that influence the stability of chlorine content in superoxidised water.

Table 2 Compares two containers and two storage conditions (with light and without light) using the ANOVA software

Concentration					
	Sum of squares	df	Mean square	F	Sig
Between groups	0.133	3	0.044	3.216	0.03
Within groups	0.77	56	0.014		
Total	0.902	59			

H^0: there is no significant difference between each factor that influences the stability of superoxidised water.

H^1: there are significant differences between each group that could influence the stability of superoxidised water.

The similarity between the result that is obtained in this experiment and the result that is done by previous research is that the chlorine degradation of superoxidised water is more rapid when the solution is exposed to sunlight. According to the result obtained by Rossi-Fedele et al.[26] the degradation of the chlorine content that has little volume (125 ml) is more rapid than the one that has a higher volume (250 ml).

On the other hand, some studies have reported a positive correlation between chlorine concentration and microbial activity in SOW.[27] Koseki et al.[28] found that SOW with a chlorine concentration of 200 mg/L was more effective in reducing the viability of *Bacillus subtilis* spores than SOW with a lower chlorine concentration (50 mg/L). Li et al.[29] reported that SOW with a chlorine concentration of 100 mg/L was more effective in reducing the viability of *Vibrio parahaemolyticus* than SOW with a lower chlorine concentration (50 mg/L). The conflicting results may be attributed to various factors, such as the type and concentration of microorganisms, the exposure time, and the pH and temperature of the SOW. Moreover, the presence of organic matter and other impurities may affect the biocidal activity of SOW and interfere with the chlorine concentration-microbial activity relationship.

The highest chlorine and lowest bulk decay constant reaction can be observed in Table 3. The higher reaction was observed in plastic bottle/light condition, and the lowest is in a glass bottle/without light, 1.6096 week^{-1} and 0.9808 week^{-1}, respectively, for the 4-week treatment. Compared to García-Ávila et al.[21] a K value of 0.154 h^{-1} was observed. It reported that the K value would be influenced by several parameters such as temperature, organic matter content and environmental condition. As presented, chlorine decay is affected by the organic matter in the water.

Several studies have investigated chlorine concentration's effect on SOW's biocidal activity against various microorganisms, including bacteria, viruses, and fungi.[30] For example, SOW with a chlorine concentration of 50 mg/L effectively reduced the viability of *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* by more than 99.9% within 30 seconds of exposure. This finding is significant in food industries as microbe activities will increase the spoilage of their products. However, increasing the chlorine concentration to 100 mg/L did not significantly improve the biocidal activity. Similarly, a study by Baek et al.[31] reported that SOW with a chlorine concentration of 50 mg/L effectively reduced the viability of *Salmonella enterica* and *Listeria monocytogenes* by more than 99.9% within 1 minute of exposure, while higher chlorine concentrations did not provide any additional benefit.

Table 3 Chlorine constant reaction rate that influences by light intensity and type of container

Weeks	Chlorine constant reaction rate, in week^{-1}			
	With light		Without light	
	Glass bottle	Plastic bottle	Glass bottle	Plastic bottle
1	0.4057	0.8759	0.0872	0.3450
2	0.8759	1.5690	0.4700	0.6931
3	1.2910	1.7928	0.6931	0.8959
4	1.6094	2.0794	0.9808	1.0989

Conclusion

Super oxidised water is a disinfectant with chlorine compound as its anti-microbial agent. It has many benefits compared to the standard disinfectant regarding health, anti-microbial activity, and environmental factors. In this experiment, the factors that influence the stability of superoxidised water in terms of chlorine stability were studied. Two distinct characteristics were used to determine the chlorine stability of superoxidised water: storage condition and type of container.

It was proven that sunlight and storage conditions are prominent in stabilising the chlorine concentration of superoxidised water. Overall, this research concludes that the type of container and storage condition are two factors that influence the stability of superoxidised water in terms of chlorine stability. The best way to keep the chlorine stability of superoxidised water is to keep it in the dark place without sunlight and use a glass container.

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None.

Conflicts of interest

The authors declare that they don't have any conflicts of interest

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