Performance of Stored Electrolyzed Seawater for Disinfection of *Pseudomonas aeruginosa*

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Abstract. Electrolysis of seawater has the ability to produce free chlorine (FC), sodium hypochlorite (NaClO), hypochlorous acid (HOCl) and other oxidants which enable it to kill pathogens making it a viable disinfectant. However, the stability of this disinfectant is somewhat unpredictable during storage. This study determined the effects of storage time on the stability of electrolyzed seawater (ESW) in terms of its physicochemical properties, specifically pH, oxidation-reduction potential (ORP), and free chlorine concentration (FCC) for 83 days and evaluated its disinfection performance on *Pseudomonas aeruginosa*. The effect of dilution in the disinfection was also investigated at ESW to water ratio of 1:0, 1:1, and 3:6. Pre-filtered seawater was electrolyzed at 1.5 A for 20 minutes using platinum-coated titanium mesh electrodes. The ESW was transferred to opaque high-density polyethylene (HDPE) bottles stored at ambient temperature without direct exposure to sunlight. The physicochemical properties of ESW at different storage times were determined, and its disinfection capability against *P. aeruginosa* was evaluated. This study has made use of two trials for the physicochemical test due to two different batches of seawater were taken. pH was found to increase over time, having a range of 6.32 to 7.705. The ORP and FCC, on the other hand, decreased over time. In accordance with the literature found, the pH ranges show that hypochlorous acid is the main driver of disinfection. Complete disinfection of ESW at 3:6 dilution was observed until 48 days of storage, while the 1:0 dilution was until 83 days and 1:1 dilution was until 51 days. FC decay (k=−0.1449 day⁻¹ and k=−0.0544 day⁻¹) was determined using Chick’s Law. Even at different dilution levels, ESW still proved its bactericidal efficacy in this research.

1. Introduction

*Pseudomonas aeruginosa* is a ubiquitous and opportunistic pathogen that naturally grows in water sources such as lakes, rivers, and coastal marine habitats (Hardalo, 1997). The proliferation and spread of *P. aeruginosa* can be problematic due to the species’ inherent resistance to many drug classes, its ability to develop resilience, rapid growth, and a recurring role in human infections (Livermore, 2002).

Disinfection plays a crucial role in preventing the spread of disease and in removing pathogens from water. Cleaning or disinfecting water began during ancient times by merely boiling water or placing objects in it. Modern methods, on the other hand, include the use of ultraviolet (UV) light, techniques such as chlorination as well as the use of ozone are also being practiced (Hendricks,
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2011). However, there are always drawbacks to each particular process. These include high capital cost, availability, and the presence of residual toxins.

While ozone and UV disinfection are generally more effective than chlorination, these methods are best for primary disinfection due to numerous factors as these methods can be costly in terms of equipment and use. Ozone, for example, can cost up to $0.68 per 1000 gallons as compared to $0.13 per 1000 gal using sodium hypochlorite (Raudales et al., 2017). In the case of drinking water, secondary disinfection where residual chlorine is added after primary disinfection to maintain a disinfection residual within the water distribution system. Thus, aside from its more extended effectiveness, chlorination is still the most used method as it is one of the cheaper alternatives for disinfection.

Despite the ease in employing chlorination for daily disinfection, there are cases wherein the reagents needed for chlorination, such as sodium hypochlorite or liquefied chlorine gas, are unavailable for use. Thus, to provide an immediate source of chlorine gas or hypochlorite ions, electrolyzed seawater (ESW) can be an alternative to standard chlorination practices. The use of ESW can be compared to chlorination because the compounds used for disinfection, mainly HOCI and OCI, are the same. As proven by Kasai et al., 2011; Oh, et al., 2010a, 2010b; Katayose et al., 2007; Damalerio 2019; ESW can be used as a disinfectant. The disinfection ability of ESW can be attributed to more than eighty elements, most notably chlorine, iodine, and bromine, that are dissolved in seawater. It is through electrolysis that compounds which have a detrimental effect on bacterial growth such as HOCl and HOBr are formed (Katayose et al., 2007). Aside from it being a cheaper alternative, the advantages of employing electrochemical disinfection include on-site production, raw material availability, and environmentally-friendly process. Electrochemical disinfection, using electrolyzed water (EW), has already been tested on several instances such as microbes on shrimp, wood and plastic boards, agricultural and food industry, etc. (Xie, 2012; Hsu and Kao, 2004; Nisola et al., 2010).

However, multiple studies have mainly focused on the immediate use of ESW or EW as alternative disinfectants, not applying the prolonged effect of time on ESW as a factor. The stability of ESW as a disinfectant over long periods of storage is still being investigated and will be the main focus of this thesis. Stability is achieved when the residual oxidants or free chlorine remains to be the dominant component in the disinfectant throughout its storage (Damalerio, 2019). Determining the stability of the ESW over time can be achieved through investigating the relationship of physicochemical properties such as the pH, Oxidation-Reduction Potential (ORP), and free chlorine concentration (FCC) with time. The physicochemical properties of the disinfectant can then be compared with actual disinfection results to determine the stability of ESW as a disinfectant.

2. Materials and Methods

2.1. Materials

Glassware, HDPE bottles, platinum-titanium electrodes, nutrient agar, N, N-dimethyl-p-phenylenediamine (DPD) powder, phosphate-buffer solution, P. aeruginosa culture, pH meter, ORP meter, and UV-Visible spectrophotometer used were acquired from De La Salle University or from local suppliers. Two batches of seawater were acquired for this study. The first batch was from Masasa Beach, Batangas, Philippines. While the second batch was from San Juan, La Union. The seawater was filtered using a PM10 microfilter before storage at ambient room temperature.

This study required forty (40) pieces of 250-mL opaque high-density polyethylene (HDPE) bottles, one 4-L beaker, one 2-L beaker, two platinum-mesh titanium electrodes, Teflon tape, and two rubber. N, N-dimethyl-p-phenylenediamine (DPD) powder, nutrient agar powder, and phosphate buffer were also employed for this study and were retrieved from local sources. Seawater was collected ten meters away from Philippine beaches, specifically Masasa Beach, Batangas, and San Juan, La Union and was filtered using PM10 microfilter.
2.2. Electrolysis Setup
The titanium-platinum mesh electrodes (50 x 192 mm; mesh size: 10 x 5 mm; electrode surface area = 23 cm²) were placed under sonication for 30 minutes to ensure that no foreign particles would be present on the surface. The electrodes were taped together with a rubber (50 x 5 x 10 mm) in between to ensure that there is a 0.5 cm gap and were immersed in the 4-L beaker containing seawater. Attached to the electrodes are wires that were connected to a power source supplying alternating current (AC).

2.3. Electrolysis of Seawater
The 4-L beaker with seawater and a current of 1.5 amperes was allowed to flow through the solution for 20 minutes. These parameters were pre-determined during preliminary experiments and were based on the physicochemical properties: pH, oxidation-reduction potential, and free chlorine concentration.

2.4. Storage Experiment
From the electrolyzed seawater, 100 mL of the solution was retrieved and placed into a high-density polyethylene (HDPE) bottle for each batch. The HDPE bottles were stored in a locker in the laboratory away from direct sunlight and at ambient temperature.

2.5. Preparation of the Working Culture of P. aeruginosa
A purified stock of P. aeruginosa culture on nutrient agar was cultivated every two weeks. This was then placed in a refrigerator that had a temperature of 4 to 10°C for the duration of the study. Prior to the disinfection experiment, a standard bacterial suspension was prepared from the stock culture for each testing day. This was done by inoculating fresh colonies of P. aeruginosa in sterile distilled water until the turbidity was according to McFarland Standard #0.5, which approximates 1.5 x 10⁸ log CFU/mL.

2.6. Disinfection Experiment

2.6.1. Preparation of Diluted Electrolyzed Seawater
The diluted electrolyzed seawater solutions were prepared in three different levels: 1:0, 1:1, 3:6. The left-hand side represents the amount of electrolyzed seawater in ratio to the amount of sterilized distilled water,

2.6.2. Disinfection of Pseudomonas aeruginosa
The disinfection experiment proceeded on the adjusted bacterial suspension of P. aeruginosa. The sample was exposed to 9 mL of diluted ESW solution (1:0, 1:1, 3:6) for a minute, followed by 9 mL of phosphate buffer solution for three (3) minutes, and lastly, 9 mL of sterile distilled water. Afterward, a standard plate count was conducted at the phosphate buffer and the sterile distilled water stage to determine whether ESW is an effective disinfectant towards P. aeruginosa even at different dilution levels. This was compared to the control group whereas the bacterial sample underwent a serial dilution of pure sterile distilled water for six dilutions. A standard plate count was done from the fourth to sixth dilution. This study had a similar setup to Damalerio’s (2019), except for the different microbe that was utilized and the use of diluted electrolyzed seawater.

2.7. Stability Experiments
The ESW solutions were evaluated for its pH, ORP, and FCC to determine its stability effect. A probe was used to evaluate the pH and ORP levels wherein the probe was dipped into the ESW. The DPD method was utilized for data collection for FCC. This is where the solution was diluted with distilled water and mixed with DPD. Afterward, 3 mL of the prepared solution was placed into a cuvette and the absorbance was determined using a UV spectrophotometer.

3. Theoretical Framework
Since there is limited literature on the topic of stored electrolyzed seawater and that when electrolyzed, the ESW contains other bactericidal compounds derived from the 70+ elements present in seawater,
these compounds can be simply reported as part of the available chlorine content. In other words, this study will only consider the NaCl content of seawater and assume that only chlorine compounds play a role in disinfection. The equations below show the anode and cathode reactions during electrolysis.

\[
2\text{H}_2\text{O} \rightarrow \text{O}_2 + 4\text{H}^+ + 4\text{e}^- \quad E_o = 1.23 \text{ V} \tag{3.1}
\]

\[
2\text{Cl}^- \leftrightarrow 2\text{e}^- + \text{Cl}_2(g) \quad E_o = 1.36 \text{ V} \tag{3.2}
\]

\[
2\text{H}_2\text{O} + 2\text{e}^- \rightarrow 2\text{OH}^- + \text{H}_2(g) \quad E_o = -0.83 \text{ V} \tag{3.3}
\]

Water molecules and chloride ions are oxidized to form oxygen, hydrogen, and chlorine gas in separate reactions. Aqueous chlorine is then formed from gaseous chlorine. This partially dissociates into hypochlorous acid, chloride and hydrogen ions. The hypochlorous acid can then partially dissociate into hypochlorite and another hydrogen ion based on the solution pH and addition of more chlorine gas. The equations for these reactions are shown below.

\[
\text{Cl}_2(aq) + \text{H}_2\text{O} \leftrightarrow \text{HOCl} + \text{Cl}^- + \text{H}^+ \quad K_H = 4\times10^{-4} \text{ at 25}^{\circ}C \tag{3.4}
\]

\[
\text{HOCl} \leftrightarrow \text{OCl}^- + \text{H}^+ \quad K_H = 3.0\times10^{-8} \text{ at 25}^{\circ}C \tag{3.5}
\]

Using the form of an equilibrium constant expression, a simplified equation that relates pH and the ion concentrations is as follows (Sincero and Sincero, 2002)

\[
[\text{Cl}_2(aq)][\text{HOCl}] = 10^{pK_H-pH+\log[\text{Cl}^-]} \tag{3.6}
\]

The form of chlorine in an ESW solution can be in the form of aqueous chlorine, hypochlorous acid, or hypochlorite ions. The form of chlorine depends on the solution pH. It is observed that aqueous chlorine is present in highly acidic conditions. As the solution of the pH increases and approaches 4.0, hypochlorous acid becomes the dominant compound. As the solution then approaches a pH of 7.5, hypochlorite dominates. The compound in focus for this study is hypochlorous acid as it is the main driver of disinfection.

4. Results and Discussion

4.1. Electrolysis Parameter Selection

4.1.1. Determination of Electrolysis Current

To determine the current used for electrolysis of this experiment, three trials were conducted under varying currents, specifically, 1.5 A, 2.0 A, and 2.5 A. In comparison with (Damalerio, 2019), she used currents 0.5 A, 1.0 A, and 1.5 A. For each electrolysis trial, the total run time was 10 minutes and samples were collected and transferred into a high-density polyethylene bottle every 2 minutes starting from the 0th minute. Two trials were done for each sample, and three physicochemical tests were determined: pH, ORP, and FCC levels.

Figure 1(a) showed two trends, for 1.5 A, pH decreases as time and current increases and was caused by the steady production of hydrogen ions while for 2.0 A and 2.5 A, pH decreased but gradually became basic. This may be due to the increase in the production of chlorides at a higher current (Hsu, 2015). Results for the 1.5 A current were similar to that of (Damalerio, 2019) and was chosen to be the optimum electrolysis current to be used since it was also the current to produce the most acidic (pH = 4.33) ESW.
4.1.2. Determination of Electrolysis Time

In the determination of the electrolysis time, four liters of seawater is electrolyzed at the determined current of 1.5 A with the duration extended up to 30 minutes. During electrolysis, 100 ml samples of ESW are collected at time intervals of 2, 4, 6, 8, 10, 15, 20, 25, and 30 minutes and placed in HDPE bottles.

It can be seen in Figure 2(a) that the pH decreases with respect to time with 2.63 being the lowest pH reading at 20 minutes which corresponds to the maximum concentration of [H+] ions before slightly increasing as time continues which may be caused by the manifestation of a reversible reaction. These aforementioned reasons are also applicable to the trend exhibited by the ORP as shown in Figure 2(b) with incremental increases until achieving stability at 20 minutes with an ORP value of 922.5 mV.

In terms of free chlorine concentration, the graph represented in Figure 2(c) shows that the FC concentration generally increases as time increases with stability being achieved at 20 minutes. This behavior can be linked back to the transformation of aqueous chlorine to hypochlorous acid at maximum [H+] concentration.

Figure 1. Results of ten minutes of ESW conducted at 1.5 A, 2.0 A, and 2.5 A for: (a) pH; (b) oxidation-reduction potential (ORP); and (c) free chlorine concentration (FCC)
4.2. Storage Effect on Physicochemical Properties
Since two batches of seawater were retrieved for this study, there are two sets of results prepared for the physicochemical properties portion. However, only one batch was necessary for the disinfection experiment since it was only necessary to see whether electrolyzed seawater may be used as an alternative source.

4.2.1. Effect on pH
The collected pH over the storage days can be seen in Figure 3, where it can be seen in the graph. The sample started out being slightly acidic. Over time, this grew to become neutral then somewhat alkaline. The electrolyzed seawater had a pH range of 6.32-7.705, with an average pH of 7.368 over 83 days. With this pH value, it indicates that hypochlorous acid is the principal component found in the solution. This suggests that the chlorine present during the first days diminished the longer it was stored. This is aligned with the studies of Damalerio (2019), Nisola et al. (2010), Cui et al. (2008), and Hsu & Kao (2004).
4.2.2. Effect on ORP
With chlorine decreasing as storage time increases, it is expected that the oxidation-reduction potential will decrease. This was proven with the initial ORP reading of 876.5 mV and the final reading of 753 mV. The effects can be seen in Figure 4. The lower ORP value is also suggestive of the decline of hypochlorous acid and a rise in hypochlorite, as well as the increase of [OH⁻] concentration. Having a positive ORP value suggests that the ESW solution oxidizes. This aligns with the results of Hsu’s (2003) and Cui’s (2009) experiments.

4.2.3. Effect on FCC
Results for the initial observation of free chlorine concentration gave a value of 115.1942 parts per million (ppm), and the final was 0.582 ppm. This showed a decline in the available chlorine as time passed as shown in Figure 5, which can be attributed to the loss of hypochlorite and hypochlorous acid. This decline can also be seen in Damalerio’s study (2019), where ESW stored in glass and plastic bottles both experienced first-order decay. According to Nisola et al. (2010), the degree of FCC decay can be caused by the sole decomposition of HOCl over time.

The decay kinetics behavior was determined in two sets and was based on the amount of chlorine present in accordance with Chick’s Law on first-order decay kinetics. For the first half of the stability experiment, the kinetic decay constant was determined to be $k = -0.1449 \text{ day}^{-1}$. While the kinetic decay constant for the second half was determined to be $k = -0.0544 \text{ day}^{-1}$. The
calculations were done separately to ensure accuracy in results since the break between the two batches affects the behavior greatly.

\[ y = -0.1449x + 1.2745 \]
\[ R^2 = 0.8993 \]

\[ y = -0.0544x - 0.625 \]
\[ R^2 = 0.5747 \]

4.3 Storage Effect on Disinfection Performance

The disinfection experimentation of \( P. \ aeruginosa \) was done for 83 days with a hiatus beginning on the 23\(^{rd} \) day and ending on the 47\(^{th} \) day due to holidays and breaks. Cell growth was considered if it falls under the range of 30-300 log CFU/mL. If the cell count is less than 30 CFU/mL this will be regarded as an absence of growth while a cell count of more than 300 CFU/mL will still be considered as too many to count. Results have shown that there was no growth until the 48\(^{th} \) storage day; however, contamination could be found in the first batch, contamination being a growth but not of the specific microbe \( P. \ aeruginosa \). Since there is an absence of the pathogen, this shows a successful result of disinfection, specifically cidal disinfection. The findings coincide with that of Damalerio (2019) and Cui et al. (2009), with their studies being until the 30\(^{th} \) storage day.

As for the pure electrolyzed seawater (1:0 dilution), \( Pseudomonas \) aeruginosa began growing on the last testing day, precisely the 83\(^{rd} \) day. Growth for the 1:1 dilution started on the 51\(^{st} \) day. However, this could not be adequately counted since it went over the 300 log CFU/mL count. There
was growth in the second trial for both the second and third dilution, namely the buffer and sterilized distilled water. Moving on to the second batch of test days, this began on the 48th day. There was growth for the 3:6 dilution for both runs of the second trial originating from the third test tube, specifically that containing sterilized distilled water. This, however, could not be counted since it reached over the limit of 300 log CFU/mL. A summary of the presence of the growth of *Pseudomonas aeruginosa* can be seen in Table 1. Table C.1 shows a more detailed summary of the growth of *Pseudomonas aeruginosa*.

### Table 1. Presence of Growth on Respective Days for Corresponding Dilution Levels

| Dilution Level | Presence of Growth on Day |
|----------------|--------------------------|
| 1:0            | 83                       |
| 1:1            | 51, 58, 83               |
| 3:6            | 48, 51, 58, 62, 83       |

5. Conclusion

This study made use of the electrolysis of seawater with a current of 1.5 Amperes for 20 minutes to produce electrolyzed seawater (ESW). The aforementioned current and electrolysis time were chosen through preliminary tests prior to the main experiment itself. The produced ESW was stored up to 83 days inside high-density polyethylene (HDPE) bottles without any exposure to sunlight and at ambient temperature. Select storage days were selected and the ESW was examined for its stability and disinfection capabilities.

Physicochemical properties of ESW namely pH, oxidation-reduction potential (ORP), and free chlorine concentration (FCC) were selected for its bactericidal proficiency and were used as the basis for the stability experiments. The increase in pH and decrease in ORP and FCC are due to a decreasing amount of hypochlorous acid through time. According to literature, the FCC should follow the first-order decay due to the decomposition of HOCl. However, this study was not able to fully show this decaying order. This could be due to factors that may have affected readings and growth, such as technical errors, old bacteria culture, or simply human error.

The disinfection experiment, on the other hand, has determined that ESW retained its bactericidal capabilities until the 48th storage day for the 3:6 dilution, 51st day for the 1:1 dilution, and 83rd day for pure ESW (1:0). It was at these dates that the first considerable growth of *P. aeruginosa* was observed. The growth of the bacterium was considered in accordance with the standard practice where a minimum cell count of 30 log CFU/ml is required for growth to be considered. For instances where a cell count of more than 300 log CFU/ml was reached, growth was simply numbered at greater than 300. With this, it is clear that the most effective ESW dilution was that of pure ESW.

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