Development of a field enhanced photocatalytic device for biocide of coliform bacteria

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ABSTRACT

A field enhanced flow reactor using bias assisted photocatalysis was developed for bacterial disinfection in lab-synthesized and natural waters. The reactor provided complete inactivation of contaminated waters with flow rates of 50 mL/min. The device consisted of titanium dioxide nanotube arrays, with an externally applied bias of up to 6 V. Light intensity, applied voltage, background electrolytes and bacteria concentration were all found to impact the device performance. Complete inactivation of Escherichia coli W3110 (~8 × 10³ CFU/mL) occurred in 15 sec in the reactor irradiated at 25 mW/cm² with an applied voltage of 4 V in a 100 ppm NaCl solution. Real world testing was conducted using source water from Emigration Creek in Salt Lake City, Utah. Disinfection of natural creek water proved more challenging, providing complete bacterial inactivation after 25 sec at 6 V. A reduction in bactericidal efficacy was attributed to the presence of inorganic and organic species, as well as the increase in robustness of natural bacteria.

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Introduction

According to the Outdoor Industry Association, more than 140 million Americans make outdoor recreation a priority in their daily lives, including 82% of Utah residents (~2.4 million people) (Outdoor Industry Association, 2012). Much of Utah’s outdoor recreation takes place in remote locations, making the consumption of water from natural sources more desirable than carrying excessive amounts of water; however, natural sources have the potential to contain bacteriological contaminants such as Escherichia coli, harmful itself and an indicator of the presence of other disease-causing bacteria. Although there are a variety of point-of-use alternatives for purifying source water, they have their disadvantages. The technology described herein utilizes a solar-driven oxidation reaction to inactivate bacteria rather than chemical oxidation (extended treatment times and unpleasant taste), physical filtration (costly pumping, need to replace filters), or ultraviolet disinfection (expensive parts) in currently available commercial products. Table 1 shows the characteristics of the most popular treatment methods currently on the market, along with the SolaPur device. The photocatalytic titania used for the reaction is non-toxic and non-consumable. The device used in this study treated over 380 L without a decrease in performance indicating that the photocatalytic titania substrate will not likely require frequent replacement.

Titanium dioxide (TiO2) is a solid-state photocatalyst that produces reactive oxygen species (ROS) when exposed to ultraviolet radiation (<387 nm) (Fujishima and Honda, 1972). When TiO2 is irradiated with sunlight, the UV portion of the
However, immobilization of TiO₂ is typically preferred if the formation and biocidal activity (Rincón and Pulgarin, 2003). Electron structure could have played a role in the recombination of the TNP coated membranes. It was suggested that the support ration for the inactivation of flow reactor that combines PEC inactivation with electropo- such as backpacking. The following reports the study on a device that is ideal for portable, point-of-use applications, This research has led to the development of an economical batch reactors) or low throughput (i.e. microfluidic chambers). established due to long treatment times (typically observed in real-world setting with natural surface water.

1. Materials and methods

1.1. Device formation

The flow reactor, shown in Fig. 1, contains polylactic acid (PLA) channels (4 mm width and 4 mm height spaced approximately 8 mm apart) with a stainless steel back plate and a UV transparent polystyrene faceplate. The anodically formed photocatalytic TNAs wire (0.28 mm diameter, 1 m long) was placed within the PLA channel at a distance 0.2 mm from the back plate, with a portion exposed to allow for connection to the power supply. The titanium wire (ESPI metals, 99.7% Ti) was cut to size, ultrasonically cleaned in a 1/1 (V/V) methanol/ isopropanol solution and then chemically polished in an acetic acid solution. Anodization was performed at 30 V for 60 min in 96.5 wt.% ethylene glycol, 0.5 wt.% ammonium fluoride, and 3 wt.% deionized water using mechanical stirring and a Pt gauze (52 mesh) cathode. After anodization, samples were rinsed with methanol and ultrasonicated in deionized (DI) water. The amorphously formed nanotubes (NTs) were crystallized at 500°C for 2 hr in a reducing nitrogen and hydrogen atmosphere. The nanotubes used in the device were designed to incorporate a large overpotential, so that water splitting was not visually observed on the TNA anode with a 50 mL/min flow at 6 V. The TNA surface was examined after annealing using a scanning electron microscope (S-4800, Hitachi, Japan).

1.2. E. coli preparation

E. coli W3110 was selected as the model bacteria to evaluate inactivation efficiencies under varying parameters and conditions. Bacterial strains were pre-cultured in Luria Bertani (LB) broth at 40°C for approximately 3 hr while shaking at 200 r/min. The culture was ready when the absorbance indicated that the bacteria were near the peak of the logarithmic growth phase, or an absorbance of ~1.1 (optical density (OD₆₀₀)). The culture was diluted in the appropriate NaCl solution (10, 100, or 1000 ppm) until the concentrations were investigated. Additionally, in-situ testing was conducted at Emigration Creek at Rotary Glen Park in Salt Lake City, Utah to evaluate the efficacy of the system in a real-world setting with natural surface water.

### Table 1 – Comparison of water disinfection systems.

<table>
<thead>
<tr>
<th>Filters</th>
<th>Ultraviolet (UV)</th>
<th>Iodine/chlorine</th>
<th>SolaPur</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mechanism</td>
<td>Physically blocks agents</td>
<td>Alters cellular components</td>
<td>Chemical sterilization</td>
</tr>
<tr>
<td>Effective against</td>
<td>Bacteria, protozoa</td>
<td>Bacteria, protozoa, viruses</td>
<td>Bacteria, protozoa (some), viruses</td>
</tr>
<tr>
<td>Treatment time (&lt;per liter&gt;)</td>
<td>&lt;1 min</td>
<td>1.5 min</td>
<td>5 min to 4 hr</td>
</tr>
<tr>
<td>Investment/volume treated</td>
<td>&gt;$60/1000-5000 L</td>
<td>&gt;$90/4000 L</td>
<td>&lt;$0.50/1 L</td>
</tr>
<tr>
<td>Weight</td>
<td>&gt;10 oz</td>
<td>&gt;5 oz</td>
<td>&lt;1 oz</td>
</tr>
<tr>
<td>Disadvantages</td>
<td>Breakable components; replacement parts requirement.</td>
<td>Easily breakable; batteries requirement.</td>
<td>Poor taste; chemical expiration; consumed.</td>
</tr>
</tbody>
</table>
day conditions were approximated by 25 mW/cm². Incident avoid settling.

were well mixed before being poured through the device to an anode material were also run as controls. Bacteria samples in the absence of UV irradiation. Additionally, blank devices without experiments were performed with the various anodes in the electroporation without a photocatalytic material. Control tested with an aluminum anode to examine the effects of steel plate was used as the cathode. A flow reactor was also between 1 and 6 V was applied to the TNA and a stainless inated water was gravity fed through the flow reactor at ~50 mL/m. For field assisted experiments, an anodic potential ~1/10th of its respective full light spectrum intensity: ~3 and UV irradiation on the surface of the flow reactor was typically through device using a solar simulator with air mass (AM) 1.5.

Disinfection was conducted in the TNA containing flow through device using a solar simulator with air mass (AM) 1.5. Sunny days were simulated with 100 mW/cm² and cloudy day conditions were approximated by 25 mW/cm². Incident UV irradiation on the surface of the flow reactor was typically ~1/10th of its respective full light spectrum intensity: ~3 and ~10 mW/cm² for 25 and 100 mW/cm², respectively. Contaminated water was gravity fed through the flow reactor at ~50 mL/m. For field assisted experiments, an anodic potential between 1 and 6 V was applied to the TNA and a stainless steel plate was used as the cathode. A flow reactor was also tested with an aluminum anode to examine the effects of electroporation without a photocatalytic material. Control experiments were performed with the various anodes in the absence of UV irradiation. Additionally, blank devices without an anode material were also run as controls. Bacteria samples were well mixed before being poured through the device to avoid settling.

1.3. E. coli inactivation

Disinfection was conducted in the TNA containing flow through device using a solar simulator with air mass (AM) 1.5. Sunny days were simulated with 100 mW/cm² and cloudy day conditions were approximated by 25 mW/cm². Incident UV irradiation on the surface of the flow reactor was typically ~1/10th of its respective full light spectrum intensity: ~3 and ~10 mW/cm² for 25 and 100 mW/cm², respectively. Contaminated water was gravity fed through the flow reactor at ~50 mL/m. For field assisted experiments, an anodic potential between 1 and 6 V was applied to the TNA and a stainless steel plate was used as the cathode. A flow reactor was also tested with an aluminum anode to examine the effects of electroporation without a photocatalytic material. Control experiments were performed with the various anodes in the absence of UV irradiation. Additionally, blank devices without an anode material were also run as controls. Bacteria samples were well mixed before being poured through the device to avoid settling.

1.4. E. coli analysis

The enumeration of E. coli concentrations before and after treatment was carried out by plating 50 μL aliquots onto LB agar. The LB agar plates were incubated overnight at 37°C and colonies were visually identified and counted. Testing was performed in triplicate and an average of the results was taken as data points. Error bars are representative of the standard deviation between individual samples in each test. A paired t-test was performed to determine statistical significance at a 95% confidence level.

1.5. Natural water disinfection

Emigration Creek at Rotary Glen Park was used as a sample location for natural water disinfection as it represents a realistic source water from which one might drink while recreating in nature. Samples collected from this location were treated under natural sunlight using voltage conditions that were found to be favorable in the laboratory controlled E. coli experiments. In-situ field data collected consisted of light intensity (total and ultraviolet (UV)), temperature, oxidation-reduction potential (ORP), conductivity and pH. The water was not turbid and contained little debris so filtering was not performed. Sodium chloride was added to the system at concentrations of 10, 100 and 1000 ppm to simulate levels examined in the lab. Samples were brought to ChemTech-Ford Laboratories for enumeration of total coliform. Natural water samples were also brought back to the laboratory for testing under controlled sunlight and with the addition of NaCl. The sample water was filtered through a 0.2 μm filter to ensure no bacteria remained in solution. Laboratory grade E. coli W3110 was then diluted into the natural water and run through the flow device under 100 mW/cm². The test was also run with a spiked NaCl concentration of 100 ppm to examine the effects of additional chloride in the water and mimic previous laboratory experiments. After the gravity fed samples were treated at a flow rate of ~50 mL/m the samples were brought to ChemTech-Ford Laboratories for enumerated total coliform and E. coli analysis.

2. Results

2.1. TNA appearance

The TNA, shown in Fig. 2, exhibited well defined tubes and/or nanograss, thin tubes that collapse and bundle when removed from the anodization solution. Average nanotube dimensions were 59 nm in diameter and 3.5 μm in length.

2.2. Inactivation performance under simulated conditions

Inactivation results for different contact times within the reactor are shown in Fig. 3. The active bacteria concentration decreases at low voltages for each time of contact, but more significantly with a 60 sec contact time. A contact time of 60 sec under static conditions in the flow reactor showed a reduction of several hundred active bacteria between 0 and 2 V, dropping to 479 CFU/mL at 2 V from the original concentration of 1010 CFU/mL, but no statistical difference between these applied voltages was seen. At 4 V, no bacteria were detected. A contact time of 15 sec under dynamic conditions expressed a similar trend, but exhibiting higher values, about double of those observed for the 60 sec treatment, dropping to 790 CFU/mL at 2 V with no statistical difference between 0 and 2 V. At 3 V, no bacteria were detected. The blank devices run as controls exhibited no significant reduction or difference in active bacteria between experiments. Subsequent testing was conducted with a 15 sec contact time under dynamic conditions, as this rate is closer to desirable product flow rates.
2.3. Inactivation performance under different irradiation conditions

Reactor efficiency data under different irradiation conditions is shown in Fig. 4. No statistical difference between the 0, 25 and 100 mW/cm² conditions was observed until 3 V, where bacteria concentrations dropped to 790, 210 and 20 CFU/mL, respectively. Bacteria subjected to 3 V and 25 mW/cm² exhibited ~80% inactivation, whereas bacteria subjected to 3 V and 100 mW/cm² exhibited ~98% inactivation.

2.4. Inactivation performance with different anode materials

An Al anode was used as a control experiment to show the effects of sunlight and the role of the photocatalytic material in complete cell death. The difference between the TNA and the Al anode on bacterial inactivation is shown in Fig. 5. Initially, the Al outperforms the TNA as ~70% bacterial inactivation is achieved. Bacterial inactivation using an Al anode is attributed to the formation of hydrolysis products, but not enough radicals are formed for complete cell death. At 3 V, the TNA becomes drastically more effective, while the Al still does not show any statistical difference in its values. The Al anode consistently displays a significantly higher current than the TNA anode. The Al anode showed a drastic decrease in active bacteria at a low voltage, but was unable to completely inactivate all bacteria while the TNA anode expressed a small decrease in active bacteria at a lower voltage, but was able to achieve complete inactivation with increased voltage.

2.5. Inactivation performance under different NaCl loading conditions

Inactivation with different NaCl concentration additions to the device are shown in Fig. 6. Bacterial inactivation became more effective with higher concentrations of NaCl. A concentration of 10 ppm NaCl showed ~50% reduction at 1 V, but did not show a statistical difference in reduction between 1 and 5 V. Little reduction in active bacteria was observed between 1 and 2 V for the 100 and 1000 ppm NaCl additions, but complete inactivation was achieved at 5 and 4 V, respectively. Typical salt concentrations found in natural waters where experiments took place have been found to be approximately 50 ppm.
2.6. Inactivation performance under natural and simulated sunlight conditions

Chemical analysis of the water collected at Emigration Creek is shown in Table 2. Experiments similar to those run in the laboratory were conducted under natural conditions at Emigration Creek. Table 3 shows the results of the experiments conducted under the conditions listed in Table 2. Total coliform were enumerated as CFU/100 mL. Total coliform was shown to reduce from a starting concentration of 2300 to 50 CFU/100 mL at 6 V and with a spiked concentration of 10 ppm NaCl. A 25 sec contact time was needed in order to achieve complete inactivation without the addition of NaCl. The results from experiments conducted in the laboratory with natural water samples are shown in Table 4. Complete inactivation was observed from an initial concentration of 2100 CFU/100 mL using an applied bias of 6 V.

3. Discussion

The environment inside the reactor during treatment is unfavorable for pathogens due to both PC generation of radicals and the applied electric field. Irradiation of the TNA with light <387 nm causes bound electrons to excite into the conduction band, leaving behind a hole situated in the valence band, Eq. (1).

\[ \text{TiO}_2 + h\nu (E_g) \rightarrow \text{TiO}_2 + e^- (\text{CB}) + h^+ (\text{VB}). \]  

(1)

These holes are then free to react with adsorbed -OH and H$_2$O, generating hydroxyl radicals (-OH). The reactions, summarized in Eqs. (2) and (3), are theorized to be of great importance in biocide, as -OH has the highest oxidation power of aqueous and elemental radical species that can be generated during photocatalysis (Munter, 2001; Nie et al., 2014).

\[ \text{TiO}_2 (h^+) + \text{OH}_{ads} \rightarrow \text{TiO}_2 + ^*\text{OH} \]  

(2)

\[ \text{TiO}_2 (h^+) + \text{H}_2\text{O}_{ads} \rightarrow \text{TiO}_2 + ^*\text{OH} + \text{H}^+. \]  

(3)

The observed increase in biocidal activity with increasing light intensity was the result of the higher concentration of UV light. While the larger contribution of high energy photons causes more electron–hole pairs to be generated, it is also followed by an increase in their recombination. Recombination effects are the reason for the statistically insignificant differences in biocidal activity between the different irradiation intensities under no applied bias. To combat the reduced

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow rate</td>
<td>50 mL/min</td>
</tr>
<tr>
<td>Light intensity</td>
<td>100 mW/cm$^2$</td>
</tr>
<tr>
<td>Ultraviolet (UV)</td>
<td>12.6 mW/cm$^2$</td>
</tr>
<tr>
<td>pH</td>
<td>8.02</td>
</tr>
<tr>
<td>Conductivity</td>
<td>1186 μS</td>
</tr>
<tr>
<td>Oxidation reduction potential (ORP)</td>
<td>397 mV</td>
</tr>
<tr>
<td>Temperature</td>
<td>12.2°C</td>
</tr>
<tr>
<td>Sulfate</td>
<td>152 ppm</td>
</tr>
<tr>
<td>Nitrate</td>
<td>0.4 ppm</td>
</tr>
<tr>
<td>Nitrite</td>
<td>&lt;0.1 ppm</td>
</tr>
<tr>
<td>Chloride</td>
<td>1 ppm</td>
</tr>
</tbody>
</table>

Table 3 – Biocide results, within 10% error, from device testing Emigration Creek water under natural settings and contact time of 15 sec unless specified otherwise.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Total coliform (CFU/100 mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original</td>
<td>2300</td>
</tr>
<tr>
<td>0 V, 0 ppm NaCl</td>
<td>&gt;2400</td>
</tr>
<tr>
<td>0 V, 10 ppm NaCl</td>
<td>&gt;2400</td>
</tr>
<tr>
<td>0 V, 100 ppm NaCl</td>
<td>&gt;2400</td>
</tr>
<tr>
<td>4 V, 0 ppm NaCl</td>
<td>1100</td>
</tr>
<tr>
<td>4 V, 10 ppm NaCl</td>
<td>1300</td>
</tr>
<tr>
<td>4 V, 100 ppm NaCl</td>
<td>300</td>
</tr>
<tr>
<td>5 V, 0 ppm NaCl</td>
<td>1000</td>
</tr>
<tr>
<td>5 V, 10 ppm NaCl</td>
<td>400</td>
</tr>
<tr>
<td>5 V, 100 ppm NaCl</td>
<td>650</td>
</tr>
<tr>
<td>6 V, 0 ppm NaCl</td>
<td>490</td>
</tr>
<tr>
<td>6 V, 10 ppm NaCl</td>
<td>50</td>
</tr>
<tr>
<td>6 V, 100 ppm NaCl</td>
<td>52</td>
</tr>
<tr>
<td>6 V, 0 ppm NaCl, 25 sec reaction time</td>
<td>0</td>
</tr>
</tbody>
</table>

CFU: colony forming units.
radical efficiency due to electron–hole pair recombination, PEC can be performed.

During PEC, electrons are driven into the circuit via the nanotubes and titanium substrate to reduce their recombination rate with holes. This extended time period allows holes to directly participate in the oxidation reaction with the bacteria which is especially important as holes have a higher relative oxidation power than •OH (Munter, 2001). The abstract art shows a depiction of the oxidation process through direct (h+(VB)) or indirect processes (i.e. •OH).

However, from the data collected, it was observed that PC and low voltage PEC (<2 V) alone do not inactivate the bacteria at flow rates that are necessary for this type of device. Gram-negative bacteria, such as E. coli, produce the enzyme superoxide dismutase (SOD) in response to oxidative stress. This enzyme can transform radicals into hydrogen peroxide (\(H_2O_2\)) and molecular oxygen via Eq. (4). Bacteria then produce catalase which breaks down intracellular H\(_2\)O\(_2\) into water and oxygen (Eq. (5)) (Rincón and Pulgarín, 2003).

\[
2O_2^{-} + 2H^+ \xrightarrow{SOD} O_2 + H_2O_2
\]  
\[
H_2O_2 + H_2O \xrightarrow{Catalase} O_2 + 2H_2O.
\]

To make the device more effective without increasing the overall size or contact time with the TNA, higher voltages were applied to the system to enhance cell death through electrical stimulation (ES). While the exact mechanism of cell death via ES is still unknown, there are two main theories: (1) a direct effect, in which an electric current disrupts the integrity of the bacterial membrane or electrolysis of molecules on the cell surface and (2) an indirect effect, such as localized temperature and pH changes, or the production of electrolysis products (Asadi and Torkaman, 2014). Upon extended exposure to the continuous direct current (DC) potential, electroporation could have also occurred. Guido et al. (2012) reported the electroporation of yeast cells under the application of a DC voltage of 2 V. If electroporation is occurring, it is proposed that just enough permeation could be occurring to allow radical penetration into the cell. In this manner, complete inactivation could occur more readily as radicals can directly attack cell components without having to oxidize the cell defense enzymes or breakdown the membrane.

Increasing the irradiation intensity on the system shows little effect at <3 V as the channels are too wide and the flow is too fast for PC generated radicals to be effective on their own. However, at 3 V, enough photogenerated radicals are present to significantly increase the rate of disinfection. After an initial drop in cell density, the Al anode maintained inactivation levels between 1 and 4 V. However, the photocatalytic material plays an important role in the disinfection process as the TNA generates electron hole pairs, while the Al anode does not.

Increasing the chloride concentration was observed to require a decrease in the voltage at which inactivation was observed. These results agreed with Nie et al. (2014), who reported that chloride and dihalide ions increase bioactivity in PEC as the photogenerated holes now have a longer lifetime and can ionize chlorine through the following reaction (Eq. (6)):

\[
h^+(VB) + Cl^- \rightarrow Cl^\cdot .
\]

The chemistry of natural surface waters varies greatly depending on a number of factors such as location, temperature, human or ecological influences. Sulfates were reported to block active sites when present in the synthesized water. Alrousan et al. (2009) found that the photolytic disinfection rate was reduced when sulfate and nitrate ions were present in solution when a TiO\(_2\) film was used. Sulfates were reported to block active sites via adsorption to TiO\(_2\) surfaces (Alrousan et al., 2009), while nitrates absorb UV light. The reasoning behind the increased efficacy of the simulated sunlight experiments over those under natural lighting is ambiguous. There is a noticeable temperature increase on the system as bacteria, removed from water of ~12°C, can experience temperatures of up to 38°C during treatment; however, water run through the blank control reactor showed insignificant changes in cell counts. Bacteria levels from the original sample that was brought into the lab were slightly lower (2100 CFU/100 mL) than water that was collected directly from the stream (2300 CFU/mL). This drop in original concentration could indicate a weakening of the bacteria when it has been pulled from its natural environment. Ongoing research is addressing these challenges through device optimization to overcome the reduction in inactivation rates for natural waters.

Inactivation of E. coli in a flow reactor device using a combination of photocatalysis and electroporation occurred in both simulated and natural water. A higher voltage was needed in the natural water containing additional inorganic and organic ions. For each of the parameters evaluated, field assisted PC was more effective for bacterial inactivation than PC alone. The optimal applied bias for complete inactivation was >3 V. Increasing the NaCl concentration in solution improved the efficiency of the device, but should be limited to be 250 ppm as this is below the Environmental Protection Agency Secondary Drinking Water Regulations for chlorides in drinking water (United States Environmental Protection Agency, 2013). The implications of this technology advances the understanding of the combination of electroporation with PEC and PC oxidation of harmful constituents in surface water and could lead to more cost effective water treatment methods that could eventually replace traditional methods.

### 4. Conclusions

This research demonstrated that point-of-use devices with higher flow rates could be fabricated using TiO\(_2\) based materials...
in combination with voltage-assistance; the applied bias generated a field that weakens the oxidation defense mechanisms of the bacteria, making it more susceptible to radical attack. The study showed the following:

(1) Biocidal activity was found to occur at lower voltages when the device was exposed to higher light intensities. The higher killing capacity is due to the higher amount of UV light, stimulating the formation of more electron-hole pairs in the TNA.

(2) The presence of chloride increases biocidal activity due to the formation of chloride radicals, which assist in biocidal activity.

(3) Lower chloride concentrations in natural water samples made biocide more difficult to be completely effective under natural lighting conditions. The presence of organics in the water can also reduce biocidal efficacy.

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