Development of a Chlorine Dioxide Gas Concentration Monitoring Unit and Kinetic Analysis of the Effect of Chlorine Dioxide Treatment on Color and Microbial Content Change of Spinach

THESIS

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By

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Abstract

Analytical methods for measuring chlorine dioxide concentration in solution have been extensively researched and documented in literature. Much less common, are the techniques for measuring ClO_2 in a gaseous medium, particularly when the measurement is needed to be made continuously in real time. While acceptable spectroscopic methods are known, complications arise due to the variability of the molecular absorption cross section which must be known to determine the concentration present. Depending on the wavelength being used and the temperature of the medium, the molecular absorption cross section changes. The purpose of this study was to develop an inexpensive and robust measurement device that would accurately measure ClO_2 in real time. Without the ability to obtain highly specified light output and detection devices, extensive calibration was needed to assure the raw measurement was properly converted into a true concentration. The resolution of the device also needed to be adjusted so that the ClO_2 concentration could be determined with a high level of accuracy throughout the concentration range of interest between 1-5 mg/l. With the aid of a spectrophotometer as a means of calibration, a measurement device was developed using only a light emitting diode (LED) and a photodiode as the major components. Through several iterations the device was adjusted to obtain a measurement precision of ± 0.19 mg/l and a minimum resolution of 0.05 mg/l over the desired measurement range. The device was also shown to be capable of withstanding repeated exposure to environments containing ClO_2 concentrations between 1-12 mg/l for exposure times of over an hour without

deteriorating the measurement capabilities of the instrument. The development of a ClO_2 measurement device for accurate concentration determination is essential for the application of ClO_2 gas for sanitation of fresh produce on an industrial scale.

Fresh produce safety, especially for leafy green vegetables, has become a focal point of interest in recent years. In an effort to reduce microbial loads present on fresh produce, gaseous sanitizers are being explored because of their higher diffusivities. In this study, spinach was exposed to chlorine dioxide (ClO_2) gas, in an effort to determine concentration and exposure time combinations which yield maximum microbial reduction while causing minimal color change to the leaf. An extensive study was first performed to determine the color change, quantified as the change in hue angle, by varying ClO_2 concentrations (2.0-6.0 mg/L) and exposure times (0.5-24 min). The resulting data were organized in form of a state diagram to determine the acceptable and unacceptable product in terms of color as a result of ClO_2 treatment. On the acceptable regions of the state diagram, ClO_2 concentration-exposure time combinations were chosen for microbial inoculation studies. Log reductions as high as 2.27 units were observed while only having a minor effect on the color of the spinach leaves (2.05±0.06 mg/l for 20 min). Kinetic data were collected to determine the rate of microbial reduction and rate of color change of spinach leaves at selected ClO_2 concentrations. These results suggested that, in order to completely eliminate color damage, high concentration short-time exposure may be the only practical way to better sanitize fragile food material like spinach. Lengthy exposure times may not provide an additional microbial reduction that is capable of outweighing the additional property damage that long-time exposures cause.

Dedication

This work is dedicated to all of those who have fostered my personal development, both intellectually and emotionally. And to my family, who has always served as my source of inspiration in my pursuit of *true* lifelong happiness.

Acknowledgments

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I would like to acknowledge the significant contributions of Christopher Gecik to the development of the chlorine dioxide measurement system. For the many hours he spent helping to prepare the equipment required for this study, I would like to thank him.

I would also like to extend a thank you to Dr. George Plum for his expertise in program design using the LabVIEW development system. He had a substantial role in the development of the program required to record data produced by the chlorine dioxide measurement system. I am very appreciative of his kindness in lending equipment to the needs of the study.

Finally, I would like to thank my committee members Dr. Gonul Kaletunc, Dr. Sudhir Sastry, Dr. Ahmed Yousef, and Dr. George Plum for their work with me throughout this study. Their willingness to take the time to critique my work is greatly appreciated.

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Table of Contents

List of Tables

List of Figures

Overview

Fresh produce safety, especially for leafy green vegetables, has been a major issue in recent years. According to the Center of Disease Control and Prevention (CDC), about 48 million illnesses, and 3,000 deaths, occur each year as a result of contaminated food (CDC, 2011). Because leafy greens create additional sanitation challenges due to their geometry, surface structure, and growing conditions, improving the sanitation of these high risk foods is of utmost importance. The conditions in which produce is grown can often cause high initial microbial loads; therefore, subsequent sanitization steps are necessary to assure the safety of fresh produce for the consumer. Current sanitation approaches mainly utilizing liquid sanitizers which are not effective in preventing outbreaks of foodborne illnesses. In an effort to improve fresh produce sanitation, gaseous sanitizers are being explored because of their higher diffusivity and depth of penetration (Han et al., 2001).

In this study, chlorine dioxide (ClO_2) gas was used to sanitize spinach, in an effort to improve the efficacy of the sanitization processes. ClO_2 has been investigated for various products yielding bacterial reductions as high as 5 log for cantaloupe (Mahmoud et al., 2008) and peppers (Han et al., 2001). ClO_2 gas has also been tested on leafy green vegetables, however, due to the variability in the color degradation and microbial reductions reported, ClO_2 effectiveness on leafy greens must be investigated further (Mahmoud et al., 2007 and Sy et al., 2005). The objective of this study was to examine the kinetics of microbial degradation and color change of spinach as a function of ClO_2

concentration, so that concentration and exposure time combination can be optimized for maximum bacterial reduction and minimum quality change. A ClO_2 concentration measurement unit was built for precise and real time monitoring of ClO_2 gas during treatment of spinach within a sealed treatment chamber. Spinach samples inoculated with *E. coli* K-12 were then exposed to various ClO_2 concentration levels and exposure times. Post exposure bacterial levels determined by plate counting and the color change were analyzed to determine the kinetics parameters for bacterial reduction and color change due to ClO_2 gas treatment.

The results are utilized to develop a produce acceptability diagram in terms of color quality and microbial safety. For ClO_2 concentrations ranging from 2-6 mg/l and exposure times of up to 24 minutes, a region of minimal quality change and 2.27 log unit bacterial reductions was observed. These reductions were greater than those reported for chlorinated water which was less 1.5 log reduction on tomatoes (Bhagat et al., 2010), 1.7 and 1.2 log reductions on lettuce and cabbage, respectively (Han et al., 2001)). The results indicate that ClO_2 gas has the potential to achieve greater log reduction than those can be obtained by using liquid sanitizers without degrading visual quality (Singh et al., 2002 and Lee et al., 2004).

Objectives

1. Design a cost effective instrument to accurately measure and monitor ClO_2 gas concentration throughout the exposure.
2. Test and optimize the ability to measure ClO_2 gas concentrations over a range applied for fresh produce sanitization.
3. Investigate the kinetics of *E. coli* K-12 reduction and color change of spinach leaves during ClO_2 gas treatment in a batch system.
4. Optimize ClO_2 gas concentration and exposure time combinations to maximize microbial reduction and to minimize color change of spinach leaves.
5. Determine the relationship between the kinetics of microbial reduction and the kinetics of color degradation of spinach sanitized using ClO_2 gas.

Chapter 1: Development and testing of Chlorine Dioxide Measurement Unit

1.1 Introduction

1.1.1. Chlorine Dioxide

Chlorine dioxide (ClO_2) is a strong oxidizing agent that has been used effectively as a disinfectant. Effective in both aqueous and gaseous forms, ClO_2 is a broad spectrum antimicrobial agent that can be used to inactivate bacteria, spores, viruses, and algae (EPA Guidance Manual, 1999). It also prevents formation of biofilms and is used for removal of bio film. The EPA approved aqueous ClO_2 for use as a sanitizing rinse for uncut fruits and vegetables (Harrison, 2006). Aqueous ClO_2 is also extensively used for the treatment of public drinking water. Gaseous ClO_2 has been used to sanitize food processing equipment, hospital and dental equipment, surfaces, rooms, laboratories, and ventilation systems (Eylath et al., 2003). Applications of chlorine dioxide includes cleaning of circuit boards in electronics industry, bleaching of textile and candles in the oil industry. Chlorine dioxide has been used often to bleach paper. Chlorine dioxide can also be used against anthrax, because it is effective against spore-forming bacteria (Weis et al., 2002).

Chlorine dioxide is a synthetic, yellow or reddish-yellow gas with a 10 times higher solubility in water than chlorine (3 g/L at 25°C). It remains as a dissolved gas in aqueous solution. Chlorine dioxide has an irritating odor, similar to that of chlorine. Odor threshold concentration of chlorine dioxide is 0.1 ppm in air. According to the

current Occupational Safety and Health Administration (OSHA) permissible exposure limit (PEL) for chlorine dioxide is 0.1 ppm (0.3 mg/m^3) as an 8-hour time-weighted average (TWA) concentration [29 CFR 1910.1000, Table Z-1]. The National Institute for Occupational Safety and Health (NIOSH) has established recommended exposure limits (RELs) for chlorine dioxide of 0.3 mg/m^3 as a TWA for up to a 10-hour workday and a hour workweek and a short-term exposure limit (STEL) of 0.3 ppm (0.9 mg/m^3) [NIOSH 1992]. Furthermore, chlorine dioxide concentrations more than 10% v/v in air may explode. Therefore, ClO_2 is not allowed to be transported but produced always on site. The typical ClO_2 concentration for gas treatment of fresh produce was reported to be in the range of 0.5-10 mg/L (Trinetta et al, 2010).

1.1.2. Chlorine Dioxide Generation

Several chemicals reactions can be utilized to generate ClO_2. A number of common reactions are given below:

$$2NaClO_{2\,(s)} + Cl_{2\,(g)} \rightarrow 2ClO_{2\,(g)} + 2NaCl_{\,(s)} \quad (1.1)$$

$$2NaClO_{2\,(aq)} + Cl_{2\,(g)} \rightarrow 2ClO_{2\,(aq)} + 2NaCl_{\,(aq)} \quad (1.2)$$

$$2NaClO_2 + 2HCl + NaOCl \rightarrow 2ClO_2 + 3NaCl + H_2O \quad (1.3)$$

$$5NaClO_2 + 4HCl \rightarrow 5NaCl + 4ClO_2 + 2H_2O \quad (1.4)$$

$$NaClO_2 + acid + FeCl_3 \rightarrow ClO_2 + byproducts \quad (1.5)$$

The most common reaction utilized by ClO_2 gas generators is given by reaction 1.1. Chlorine dioxide is generated by passing approximately 2-4% chlorine (Cl_2) gas by volume in nitrogen through a cartridge containing thermally stable sodium chlorite

($NaClO_2$). Since ClO_2 generation is nearly instantaneous, this reaction allows the concentration of ClO_2 gas to be closely controlled (EPA Guidance Manual, 1999). Reaction 1.2 is typically used in a laboratory setting to produce aqueous ClO_2. Chlorine gas is bubbled through $NaClO_2$ dissolved in water creating aqueous ClO_2. Since Cl_2 is known to be harmful if inhaled, it is important to assure all Cl_2 is dissolved or captured to prevent human interaction with the gas. Despite the potential hazards associated with chlorine gas, a laboratory setting typically contains the necessary safeguards and personnel to handle this hazardous gas safely. Reactions 1.3 and 1.4 are utilized when treating drinking water or waste water. Reaction 1.3 is nearly 100% efficient, however, it has the potential to create sodium chlorate and chlorine gas if chemical concentrations are allowed to get so high as to cause the solution to become <3 pH. The production of sodium chlorate reduces the quantity of ClO_2 generated, and chlorine gas can be harmful to humans (EPA Guidance Manual, 1999). Reaction 1.4 will generate as much ClO_2 as HCl used, while the $NaClO_2$ can be added in excess without negatively impacting the reaction efficiency (Oxychem, 2009). As long as the $NaClO_2$ is added in excess, gaseous Cl_2 will not be formed, providing a safer reaction. Although the efficiency of reaction 1.4 is only 80%, this reaction is preferred when avoiding the risk of Cl_2 exposure is desired. Commercially available sachets (ICA TriNova Corporation, LLC, Newnan, GA, USA) utilizing reaction 1.5 are designed to release a precise amount of gas. Such sachets consist of two compartments, one containing a granular porous solid impregnated with sodium chlorite and the other containing a granular porous solid impregnated with acid and an acid precursor iron chloride ($FeCl_3$). Once the septum was broken in between the

two compartments, the chemicals mix together and ClO_2 is released (Mahovic et al., 2009).

1.1.3. Detection Methods

Chlorine dioxide concentration can be measured using several methods depending on whether it is being measured suspended in a liquid or as a gas. Some prevalent forms of measurement in liquid include chromatographic, amperometric and spectrophotometric methods (CDC, 2000). Chromatography utilizes ion exchange resins to separate a sample as it is pumped through a channel. The anions within the sample attach to the resin while the rest of the eluant passes through the channel. The chlorite ions residing in the channel are then measured by a detector to indirectly determine the ClO_2 concentration. Amperometric methods involve amperometric analyzers which are capable of subjecting the sample to a steady electrical current. As a particular titrant, usually phenyl arsine oxide, is added to the sample, it is reduced by the chlorine dioxide in the solution. The chlorine dioxide concentration is then determined by the amount of electrical current that is required to maintain a constant concentration of the titrant despite its reduction by chlorine dioxide. Spectrophotometric methods of ClO_2 measurement involve a color change in the liquid sample. One of the most common techniques of this kind is the N,N-diethyl-p-phenylenediamine (DPD) method (Gates, 1997). In this technique, the concentration of ClO_2 is determined by the amount of ferrous ammonium sulfate that is required to obtain a specific color change (CDC, 2000). Using the equations given in the

Standard Methods for the Examination of Water and Wastewater (1985) a measureable optical absorbance is converted into a concentration of ClO_2.

Although it is possible to dissolve gaseous ClO_2 in water to measure the concentration, direct measurement methods for gaseous ClO_2 are limited to ion chromatography and spectrophotometry. Ion chromatography can be done similarly to the detection of ClO_2 in a liquid medium by pulling a volume of air through a toxic vapor detector tube. The chemicals within the tube react with the passing ClO_2 and cause the tube to change colors so that the intensity and length of the stain can be converted into a concentration. The spectrophotometric method can be used to directly measure the ClO_2 concentration in a gaseous medium by measuring the quantity of light that passes through a gas containing ClO_2. By taking the difference in the intensity of a light source traveling across a given path length between the sample containing ClO_2, and the gaseous medium without ClO_2, the concentration can be determined (EPA, 1999).

1.1.4. Spectroscopic Measurement

Spectrophotometric measurement of ClO_2 is based on the absorbance of UV light by chlorine dioxide. The absorbance spectrum of aqueous ClO_2 exhibits a maximum UV light absorbance at approximately 360 nm. The absorbance spectrum of gaseous chlorine dioxide exhibits several minimum and maximum UV absorbance as the temperature and wavelength of the UV light used changes (Clapper and Gale, 1964). Figure 1.1 shows the molecular absorption spectra from a wavelength of 240-460 nm at three different temperatures. The molecular absorption cross section is a measurement of the ability of a

molecule to absorb a given wavelength (Wahner et al., 1987). An increase in molecular absorption cross section correlates to an increase in absorption at a given wavelength.

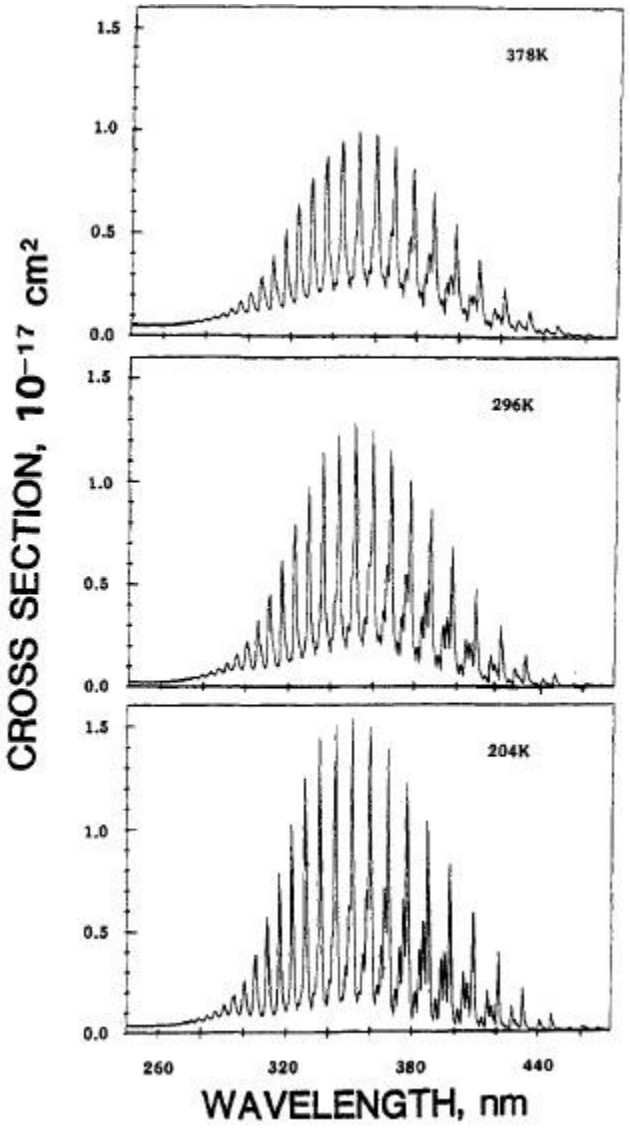

Figure 1.1. Absorption cross sections of gaseous ClO_2 (taken from Wahner et al., 1987).

Although the absorption spectrum displays a maximum absorption around 360 nm, the actual absorption values fluctuates. Therefore, the analysis of the gaseous ClO_2 spectrum is more challenging in comparison to the spectrum of aqueous ClO_2. The amount of light

absorbed over a defined path length of ClO_2 is proportional to concentration of the gas and its molar absorptivity, according the Beer-Lambert Law (equation 1.7).

$$A = \varepsilon cl \tag{1.7}$$

where A is absorbance, ε is molar absorptivity (M^{-1} cm^{-1}), c is concentration (mol/L), and l is path length (cm). The molar absorptivity of gaseous ClO_2 was reported to be 1250 M^{-1} cm^{-1} at 360 nm (Fuwa and Vallee, 1963). You should mention about some other values reported in the literature too. The absorbance measured by spectrophotometry is defined as:

$$A = -log\frac{I_m}{I_o} \tag{1.8}$$

where I_m is the measured intensity of transmitted light, and I_o is the intensity of incident light.

In order to utilize the spectroscopic technique to measure ClO_2 concentration over a large range of concentrations, several different components are needed in order to keep the absorbance reading between 0.1-0.9 A. Either the light source or the path length must be altered according to equation 1.7 to keep the absorbance measurement in a linear range. This may involve having several different light sources or several different path lengths contained within one instrument. Many commercial inline systems withdraw a sample from the system and return the sample once the measurement has been made. In this setup, gas is removed through an opening, passed across the measurement device, and returned to the system in a continuous loop. Alternatively, measurement devices can be built as standalone units that do not require to be bound to the ClO_2 source. These

units can be placed in an area where ClO_2 is present, even if the area is not sealed off, and make continuous concentration measurements (Carson et al., 2011).

1.2. Objectives

Measurement and monitoring of chlorine dioxide concentration during the treatment are vital to delivering the desired level of ClO_2, and to determine the efficacy of the ClO_2 treatment. The measurement device to monitor ClO_2 concentration should be inexpensive, accurate at the ClO_2 levels commonly used for fresh produce, and robust. The purpose of this study was to design, build, and test a ClO_2 measurement device that was both inexpensive and versatile. More specifically, the objectives were:

1. Design a cost effective instrument to accurately measure and monitor ClO_2 gas concentration.
2. Test and optimize the ability to measure ClO_2 gas concentrations over a measurement range applicable to fresh produce sanitization.

1.3. Materials and Methods

1.3.1. Materials

An LED (FoxUV TO-18; The Fox Group Inc., Pointe-Claire, QC, Canada) with a glass ball lens was used to generate the UV light in the range of 340-400 nm with a maximum intensity at 363 nm. The LED was protected from ClO_2 by a sapphire window (Edmund Optics, Barrington, NJ, USA) that was mounted in front of the UV light source. A photodiode (FGAP71; Thorlabs Inc., Newton, NJ, USA) received the light emitted by

the LED. Both electrical components were contained within an enclosure made of electrical PVC.

The current created by the intensity of the light striking the photodiode was measured as an analog voltage and digitized by an A/D converter (LabJack U3; LabJack Corporation, Lakewood, CO, USA). Key specifications of the LabJack U3 data acquisition board are given in Appendix A. Data acquisition was done using software developed with the LabVIEW development system (LabVIEW 2009; National Instruments, Austin, TX, USA).

The ClO_2 was generated inside of a 43.6 L, rectangular, sealable, polypropylene container (treatment chamber) (1600 Case; Pelican Products, Inc., Torrance, CA, USA). The samples were contained within a 4.0 L styrene-acrylonitrile, sealable container (sample chamber) (OXO, Chambersburg, PA, USA) which was mounted inside the treatment chamber. Chlorine dioxide was generated based on reaction 1.5. Sodium chlorite (RDCS0440-500B1; Ricca Chemical Comp., Arlington, TX, USA) was combined with hydrochloric acid (E484; Amresco LLC, Solon, OH, USA) to produce ClO_2.

1.3.2. Methods

1.3.2.1. Treatment chamber Setup and Components

The treatment chamber was modified to allow the power cables for the two fans and the fiber optic cable to enter. For a kinetic study in a batch system, it was necessary to prevent the samples from being exposed to ClO_2 gas prior to reaching a constant gas

concentration. Therefore, a sample chamber was constructed inside the treatment chamber to prevent the fresh produce samples from being exposed to ClO_2 gas prior to a constant concentration level was reached (figure 2.2). The sample chamber had a mechanized arm that was mounted to the wall of the treatment chamber to allow the sample chamber to be opened and closed from the outside. The arm was made of electrical PVC, and was pressed in to release the sealed lid. The arm was then pulled out to remove the sample chamber lid exposing the samples to the ClO_2 gas in the treatment chamber. Pressure release valves were not used in the treatment chamber because the pressure increase was negligible, $4.0x10^{-3}$ atm increase at a constant temperature for a maximum ClO_2 generation of $3.3x10^{-3}$ moles.

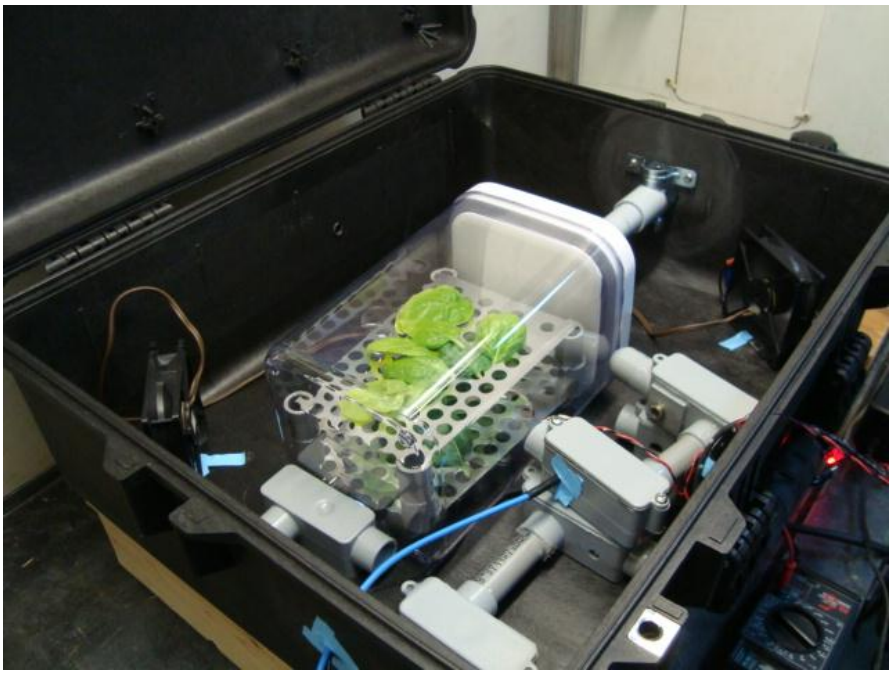

Figure 1.2. A smaller sealed sample chamber containing spinach samples inside the larger treatment chamber.

Figure 1.3 shows a schematic of the entire setup used in this study including the treatment chamber, sample chamber, data acquisition board, spectrophotometer, and computer (photograph in Appendix B).

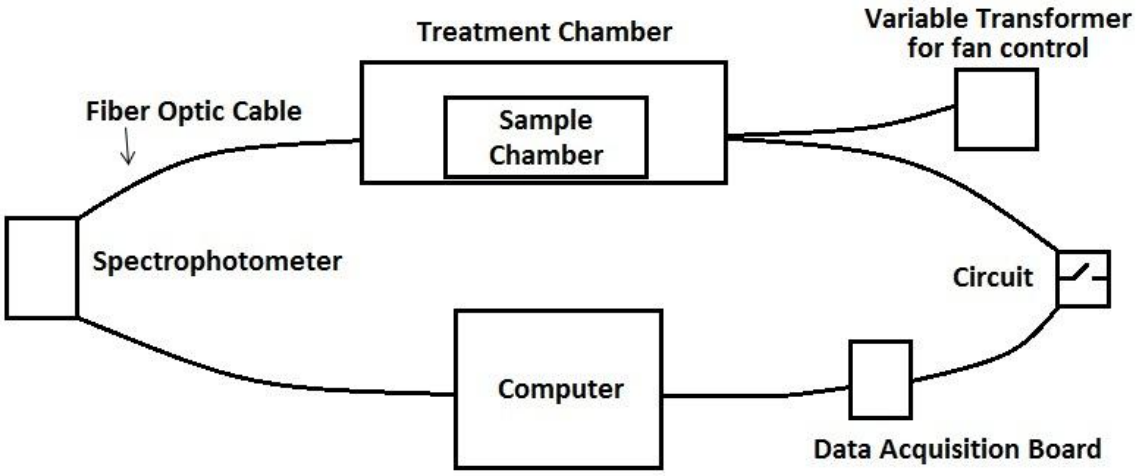

Figure 1.3. Schematics of overall ClO_2 exposure and data acquisition system.

1.3.2.2. Chlorine Dioxide Generation

Solutions of 35% $NaClO_2$ and 6N HCl were prepared. The amount of each solution was calculated based on the desired theoretical ClO_2 concentration and the stoichiometric balance of reaction 1.5 (sample calculation shown in Appendix C). Chlorine dioxide was generated by mixing the two solutions inside a 10ml beaker using a stir bar controlled by a stirrer that was placed under the treatment chamber (Cimarec 2; Thermo Fisher Scientific, Pittsburgh, PA, USA). First, an appropriate amount of the 35% $NaClO_2$ was added into the beaker. Then, immediately after the HCl addition, the

treatment chamber was closed. Once the ClO_2 concentration reached a constant value, the sample chamber was opened to bring the samples in contact with the gas for treatment. The temperature and relative humidity were varied during the treatments between 19-27 °C and 22-53%, respectively.

1.3.2.3. Chlorine Dioxide Measurement

1.3.2.3.1. Photodiode Measurement Unit

The ClO_2 measurement unit was built using electrical PVC, which does not interact with chlorine dioxide gas (Olsen et al., 2011). The LED and photodiode were set 6.1 cm apart and both housed within separate conduit boxes and were sealed using silicone (premium waterproof silicone; General Electric, Fairfield, CT, USA). The conduit boxes were connected by a piece of electrical PVC tubing sealed to be air tight using PVC cement (#30818; Oatey, Cleveland, OH, USA). A mounting column, used to protect the LED from ClO_2 gas, was screwed into a threaded plate and sealed in place with PVC cement. The sapphire window was mounted on the end of the mounting column to prevent ClO_2 from interacting with the LED and to make it easier to clean condensed ClO_2 that settled on the surface (figure 1.4).

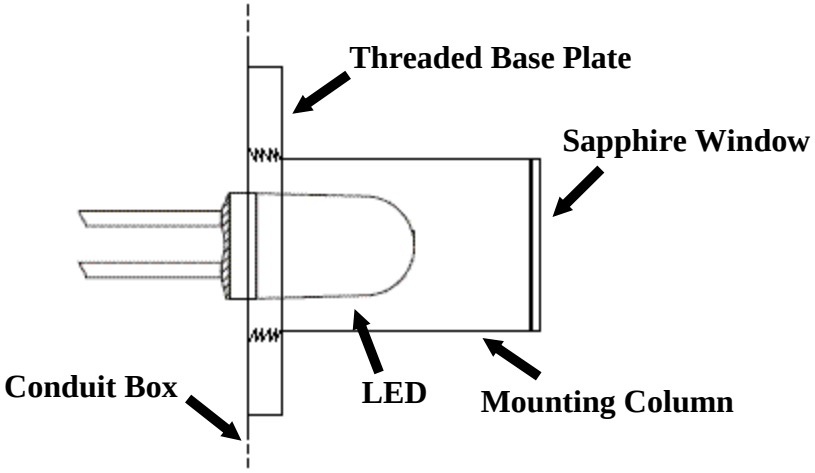

Figure 1.4. Schematics of LED section of the measurement unit.

A second system similar to the one shown in Figure 1.4 was built to house the photodiode. The photodiode was sealed in place with hot glue, and sapphire window was added to protect the active region of the photodiode.

1.3.2.3.2. Circuit Design

The circuit used for the photodiode measurement unit is shown in Figure 1.5. Component 1 was designed to control the intensity of the LEDs. By adjusting the potentiometer the current was adjusted between 5.0-30.0 mA. The intensity of the LED directly affects the amount of leakage current that will flow from the power source through the photodiode.

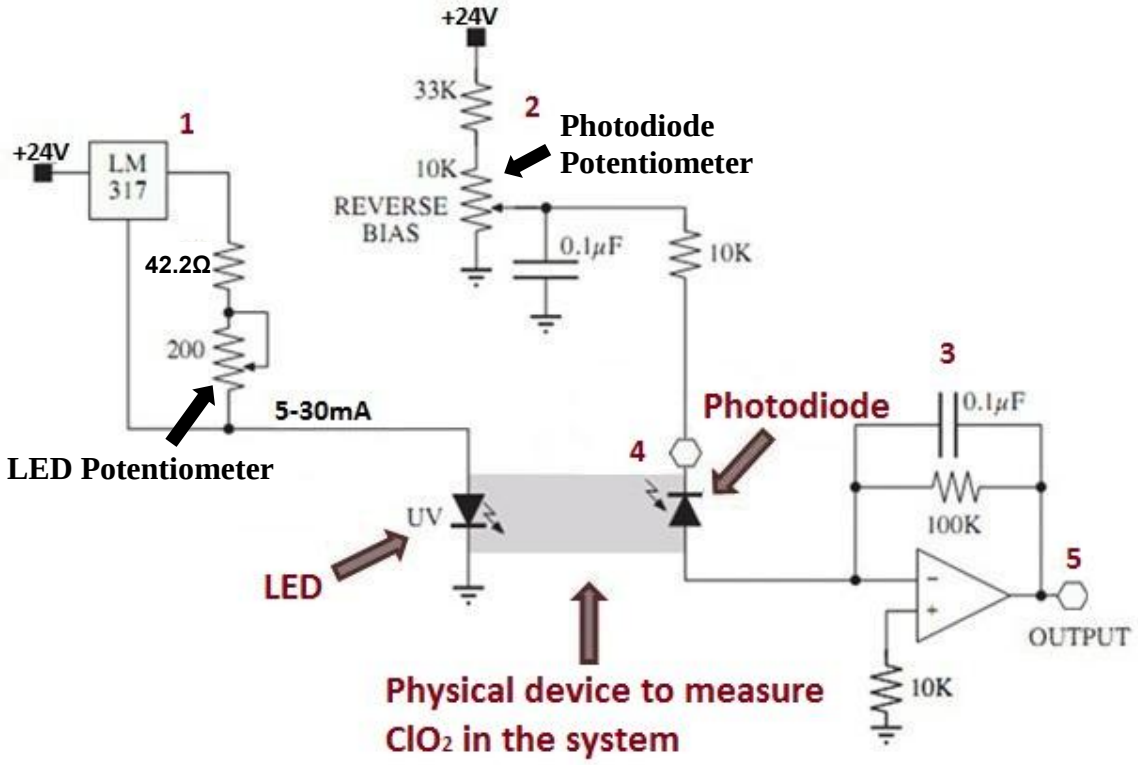

Figure 1.5. Schematics of the circuit used in the ClO_2 measurement unit.

Component 2 was included to control the reverse bias voltage within the circuit. The reverse bias was used to improve the linearity and response speed of the photodiode by decreasing the junction capacitance. However, this causes the dark current and noise to increase in the device. By varying the bias voltage between 3mV-10V, the bias voltage that impacted the output voltage the least was chosen. Varying the bias voltage from 3 to 500 mV resulted in a 7% increase in the total observable voltage range. However, varying the bias voltage from 0.5 to 10V had very little effect on the final voltage being produced. The total observable voltage range increased by less than 2% between a bias voltage of 0.5-10V when ClO_2 was not present, suggesting that the bias voltage should be

greater than 0.5 mV. Above this voltage, the affect within the final designed circuit was minimal, so a 5 V bias voltage (suggested by ThorLabs, Inc.) was used throughout all trials.

Component 3 was added to amplify the current from the photodiode so that a measureable voltage can be obtained. The amount of the resistance in parallel with the amplifier was determined from the LED data sheet (Appendix D) for a maximum power output of 700 μW when the current through the LED is 30.0 mA. Using this maximum power the responsivity of the photodiode was calculated from the following equation;

$$R_\lambda = \frac{I_P}{P} \tag{1.9}$$

where P is power (W), R_λ is responsivity (A/W), and I_P is photocurrent (A). The responsivity at any given wavelength was determined from Fig 1.6 and the maximum current passing though the photodiode was calculated (Thorlab, 2010).

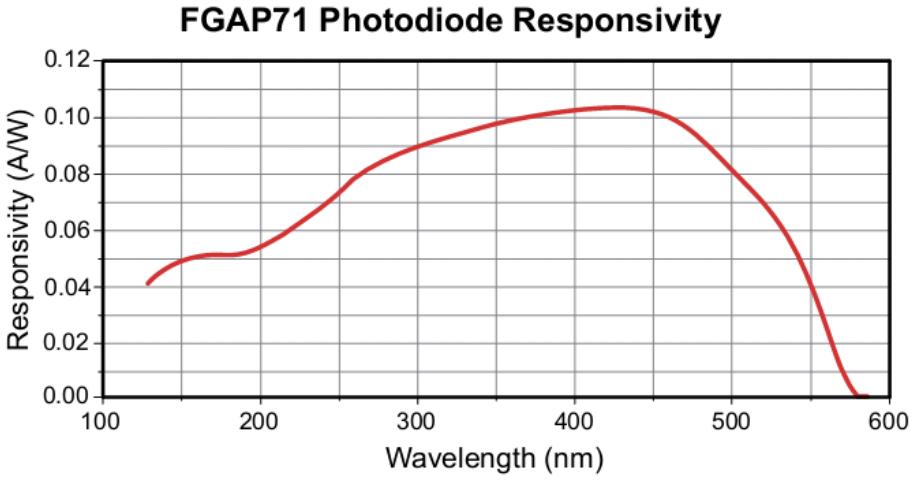

Figure 1.6. Responsivity of the photodiode as a function of the wavelength of the light source; taken from (ThorLab, 2010).

The responsivity in the vicinity of 360 nm wavelength was calculated to be 0.07 mA, suggesting that the maximum current that can pass through the photodiode and on to the amplifier is 0.07 mA, based on the assumption that 100% of the light generated by the LED is striking the active region of the photodiode. Because the LED has a 5° viewing half angle, and the LED has a radius of 2 mm, the percent of the UV light actually striking the photodiode was calculated to be 2.9% (see Appendix F for detailed calculations). The amount of power that was actually utilized was found to be 20.3 μW. The photocurrent corresponding to the calculated power was determined to be 0.00203 mA instead of 0.07 mA. The maximum resistance possible in parallel with the amplifier under these conditions was calculated to be 1.2MΩ (equation 1.10).

$$R = \frac{V}{I} = \frac{2.44V}{2.03x10^{-6}} = 1{,}201{,}970\Omega \qquad (1.10)$$

Throughout the study the voltage measurements in the absence of ClO_2 were approximately 700mV. Using a 100kΩ resistor and the measured maximum voltage, the actual current through the circuit was found to be 0.007mA; much higher than the predicted current due to the viewing angle of the LED (0.00203mA). The difference in the observed and calculated value was most likely due to the LED being more intense in the center of the beam. The LED was fixed in place where the beam produced the largest voltage increase when striking the photodiode. For this reason, only a 100 KΩ resistor was needed to create 700 mV of measureable voltage.

Component 4 indicates the photodiode measurement unit and component 5 is the voltage measurement recorded by the data acquisition board.

1.3.2.3.3. Spectroscopic measurement unit

A second measurement unit (spectroscopic measurement unit) was built identical to the photodiode measurement unit but was connected to a spectrophotometer (USB4000 Fiber Optic Spectrophotometer; Ocean Optics, Inc., Dunedin, FL, USA). The spectrophotometer received the UV light from the LED via a fiber optic cable attached to a lens mounted inside a conduit box across from the LED. The lens was clamped against the opening in the conduit box to allow the lens to be positioned at different locations as was necessary to regulate the intensity of light reaching the CCD array. The embodiments of the ClO_2 measurement instruments were constructed to assure the mounting columns were adequately sealed to prevent any ClO_2 from penetrating into the units. The final construction is shown in Figure 1.8 and Figure 1.9.

A schematic of the spectrophotometer used to measure the absorbance of ClO_2 in the system is shown in figure 1.7. Light from the LED was passed through a lens and was carried by a fiber optic cable into the instrument. The light is then diffracted and reflected as first-order spectra onto the CCD array.

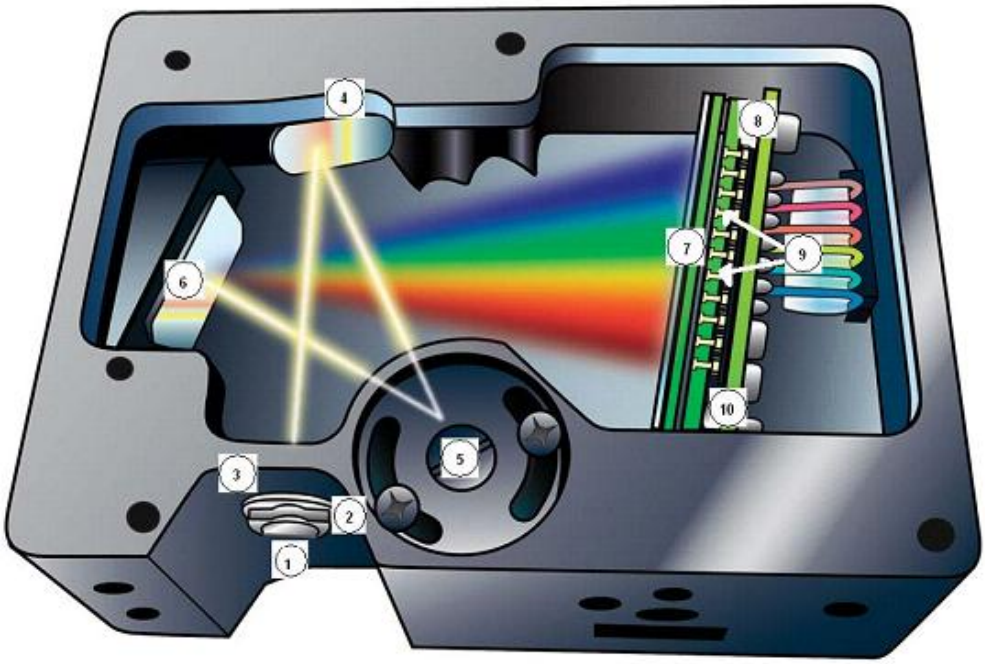

Figure 1.7. Interior view of the Ocean Optics USB4000 spectrophotometer. 1-connector (where the fiber optic cable feeds the light through a filter and slit into the instrument), 2-slit, 3-filter, 4-collimating mirror, 5-grating, 6-focusing mirror, 7-CCD array, 8-detector, 9-OFLV filter, 10-UV4 Detector (from Ocean Optics USB4000 spectrophotometer manual).

The CCD array had 3648 pixels, each responsible for detecting a wavelength within the span of 201-532 nm. For this study, a range of pixel closest to 360 nm (358.45-361.05 nm) was chosen to calculate the absorbance of the ClO_2 within the system. Each value collected within the range of pixels was then converted into an absorbance by equation 1.11.

$$A = -log\frac{I_m - I_d}{I_o - I_d} \tag{1.11}$$

where I_d is the light intensity when the LED is turned off, I_m is the light intensity measured, and I_0 is the initial light intensity where the concentration of ClO_2 is zero. As

the ClO_2 concentration rises, more of the light is absorbed causing the measured light intensity to decrease. This, in turn, causes the measured absorbance to increase. The absorbance was then determined by fitting a polynomial to the 28 pixels from 358.45-361.05 nm. From this curve, the absorbance at exactly 360 nm was found using the equation of the polynomial (Appendix G).

1.3.2.4. Measurement Unit Setup for Data Collection

The photodiode measurement unit (figure 1.8) and spectroscopic measurement unit (figure 1.9) were placed in the treatment chamber and connected to one another via a 2.5 mm stereo connector (171-0025-EX; Mouser Electronics, Inc., Mansfield, TX, USA) to assure the same current ran through both LEDs (photographs in Appendix E).

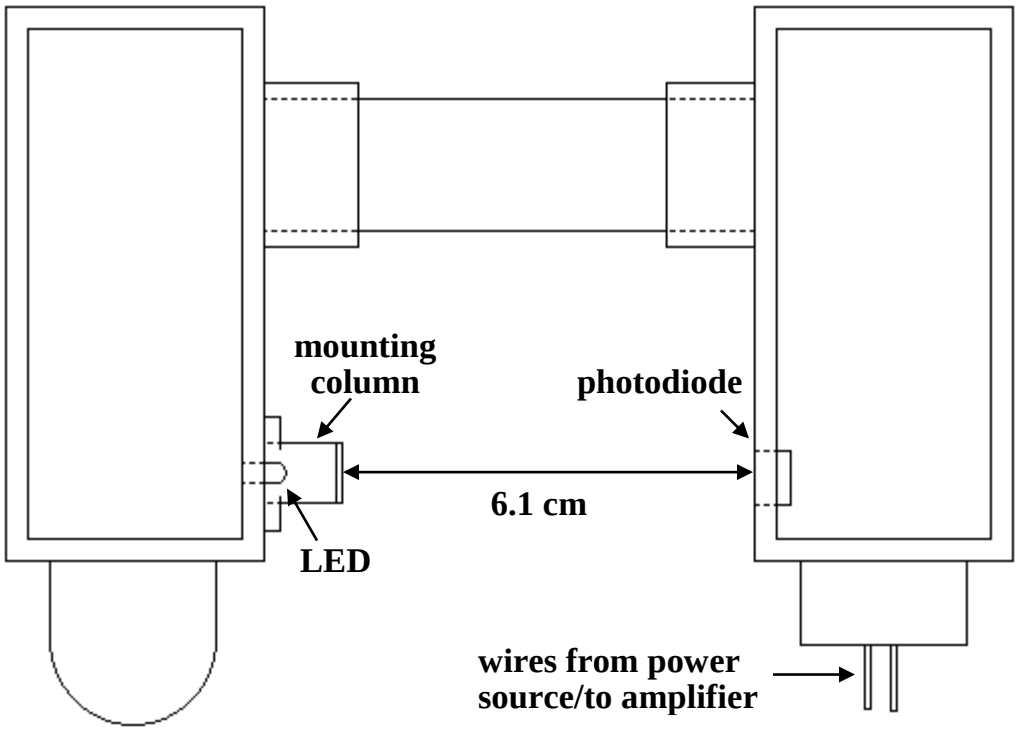

Figure 1.8. Schematics of photodiode measurement unit.

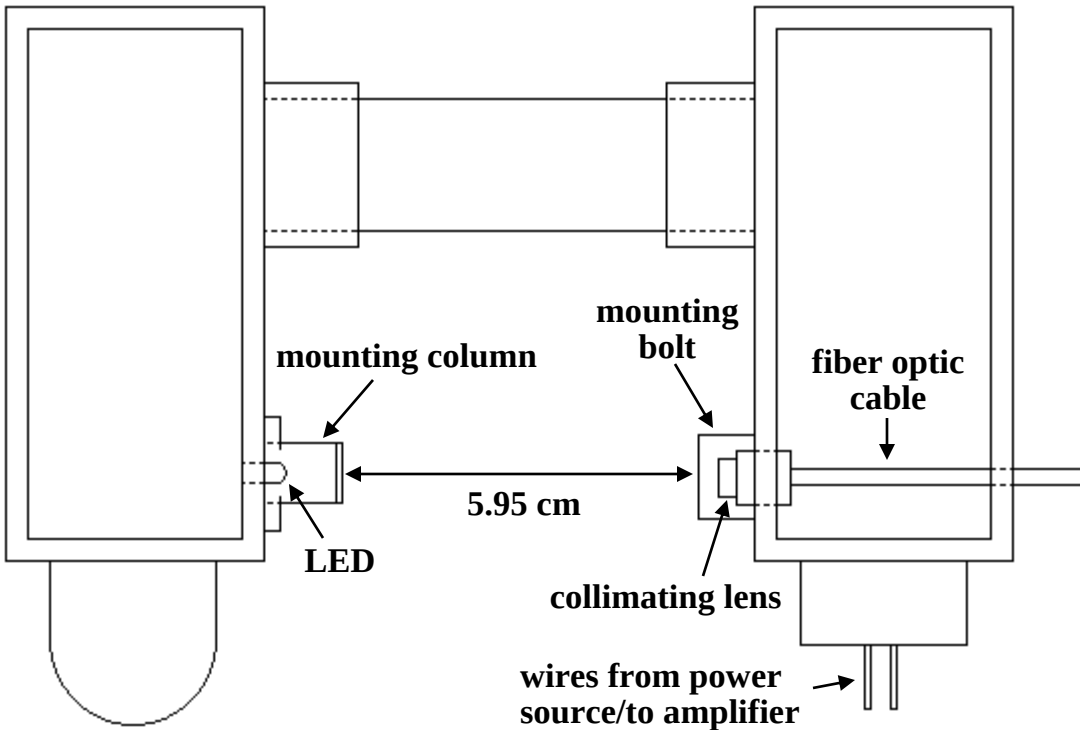

Figure 1.9. Schematics of the spectroscopic measurement unit.

The LEDs were powered so that the current running through the LEDs was stabilized around 30.0 mA. The potentiometer controlling the current through the LEDs was adjusted in order to limit the maximum current to 30.0 mA to assure the LEDs did not burn out.

Chlorine dioxide concentrations were measured several times in order to assure the reproducibility of the concentration measurement. Both the photodiode measurement unit and spectroscopic measurement unit were set up and tested at the beginning of each experiment to assure consistent initial conditions. The windows and lenses on both units were cleaned with lens paper and distilled water. The initial voltage and voltage caused by the dark current were then recorded along with the initial temperature and relative

humidity. Equation 1.12 was used to calculate absorbance from the voltage measurement:

$$A = -log\frac{V_m - V_d}{V_o - V_d} \tag{1.12}$$

where V_m is the measured voltage in presence of ClO_2, V_o is the initial voltage before ClO_2 generation, and V_d is the voltage when LED was not turned on.

The data were collected using a program written for LabVIEW. The "get spectrum" mode was used to read the intensity of the light reaching the CCD array. The "absorbance" mode was used to read the calculated absorbance at the CCD array. The intensity measured by the spectrophotometer was adjusted to be below 32,000 counts with the integration time above 3,800 μs at the beginning of each trial to prevent the pixels from become saturated and unresponsive to changes in intensity. The preferred intensity for collecting data would be the highest intensity that could be achieved without risking the intensity rising above the saturation point of the sensor. The placement of the lens within the embodiment was adjusted before being clamped in place by the bolt assuring the intensity was around 31,000 counts.

Once the intensity had been verified, the mode was set to "absorbance" measurement mode and the program was run. The LED was turned off to measure the voltage in the absence of light. The LED was then turned on and another measurement was taken to determine the initial intensity. The reactants were then mixed together in a beaker within the treatment chamber before closing the chamber and beginning the data collection. Data were collected as a function of time until a constant concentration was

reached, and for a period during the constant concentration before the LabVIEW program was terminated.

1.3.2.5. Raw Data Conversion to Actual Concentration

The voltage data collected using the photodiode measurement unit were converted to absorbance using equation 1.12 and the intensity data collected by the spectroscopic measurement unit were converted to absorbance using equation 1.11. The concentration data were calculated from absorbance values according to the Beer-Lambert Law (equation 1.7). The correlation (equation 1.14) between the concentration values from the spectroscopic measurement unit and the photodiode measurement unit was used to calculate the corrected ClO_2 concentration within the treatment chamber.

1.5. Results and Discussion

1.5.1. Preliminary Adjustments to Measurement Units

Preliminary data collected using the photodiode measurement unit are shown in Figure 1.11. Initially, the photodiode measurement unit was either too sensitive or not sensitive enough to detect CO_2 concentrations over the entire range of 1-5 mg/l. In some instances, the measurement unit was completely saturated before the steady state value was reached (Figure 1.10 shown by red circles, blue diamonds, blue squares, and purple circles). Useful data was only collected by the voltage measurement device in a small region in each of the trials between the high and the low voltage reading.

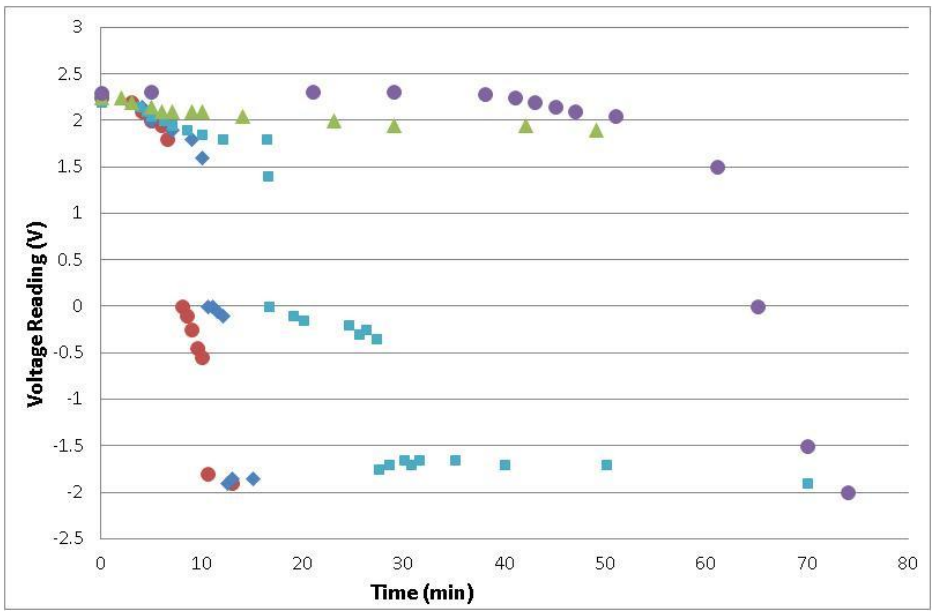

Figure 1.10. Voltage versus time readings for various ClO_2 concentrations and LED intensities. 3.0mg/l, 16.0mA (red circles); 1.5mg/l, 16.0mA (dark blue diamonds); 1.0mg/l, 16.0mA (light blue squares); 0.5mg/l, 16.0mA (green triangles); 3.0mg/l, 22.7mA (purple circles).

For instance, the sample with a theoretical concentration of 3.0 mg/l and an LED intensity of 22.7 mA did not show any detectable voltage change for the first 40 minutes of ClO_2 generation. Then, the rate of voltage change over time was too fast to obtain good resolution. It was necessary to adjust both the range and resolution of voltage readings over the desired ClO_2 concentration range. Therefore, the original design (circuit shown in Appendix H and data shown in figure 1.10) had to be simplified to remove the second amplification component from the circuit. The additional amplification component was resulting in a x10 amplification of the voltage according to equation 1.13.

$$V_{output} = V_0 * \frac{R_{A2nd}}{R_{A1st}} = V_o * \frac{1M\Omega}{100K\Omega} = 0.25V * \frac{1M\Omega}{100K\Omega} = 2.5V \qquad (1.13)$$

where V_{output} is the voltage measured after the second amplifier, V_0 is the voltage before the second amplifier, R_{A2nd} is the value of the resistor in parallel with the second amplifier (shown in Appendix H), and R_{A1st} is the resistor in parallel with the first amplifier (component 3 in figure 1.5). If the voltage through the circuit was even 0.25V, the output voltage after amplification from the second amplifier would exceed 2.44V according to equation 1.6, the highest voltage the data acquisition board could read. The most likely explanation for the results in figure 1.10 was that a small voltage being produced by the leakage current through the photodiode was being amplified to a value too large for the data acquisition board to read. To correct this problem, the intensity of the current through the photodiode had to be increased and the amplification of the signal had to be decreased (discussed in section 1.5.3.).

1.5.2. Chlorine Dioxide Generation Optimization

Reaction 1.4 was chosen to produce ClO_2 because while it is theoretically 20% less efficient than reaction 1.3, ClO_2 is generated without the production of sodium chlorate (EPA Guidance Manual, 1999). Reaction 1.3 produces sodium chlorate if the reactants are not precisely metered into the beaker where the reaction is taking place, causing a reduction in reaction efficiency. Reaction 1.4 is more reliable when adding the reactants one by one. In this case, $NaClO_2$ can be added in excess to avoid producing unwanted byproducts (OxyChem, 2009). $NaClO_2$ was always added in excess of what was theoretically needed to assure the HCl was fully reacted with the $NaClO_2$. Table 1.1

shows the rate and completeness of the reaction using different concentrations of reactants.

Table 1.1. Reaction completion time and efficiency.

Theoretical Concentration	% NaClO2 Concentration	% HCl Concentration	Excess NaClO2	Total Reactant Volume	Generation Time	Measured Concentration	Reaction Efficiency
mg/L	%	N	%	mL	min	mg/L	%
1	7.5	1	33	2.03	70	0.50	50.3
1	7.5	6	33	1.48	42	0.52	52.3
1	35	6	33	0.41	7	0.59	59.4
3	35	6	33	1.22	8	1.63	54.2
3	35	6	33	1.22	8	1.63	54.2
3	35	6	66	1.43	10	1.76	58.8
3	35	6	66	1.43	10	1.75	58.5
6	35	6	33	2.43	11.4	3.14	52.3
6	35	6	66	2.87	13.5	3.19	53.2

Chlorine dioxide was originally generated using 7.5% $NaClO_2$ and 1N HCl based on the data reported in the Millennium III AC-10 Chlorine Dioxide Generator manual (Siemens, Washington, D.C., USA) (Alarid et al., 2011). Under these conditions, a theoretical concentration of 1.0 mg/l did not reach its maximum concentration until 70 minutes of generation. Since the concentrations of reactants were low, the H_2O, both initially present and generated by the reaction, dissolved the ClO_2 gas, reducing the ClO_2 present in the atmosphere. To increase the reaction rate, a larger concentration of $NaClO_2$ and HCl were used. Because the solubility of $NaClO_2$ is 359 g/l, a $NaClO_2$ concentration of

35% (350 g/l) $NaClO_2$ (w/v) and 6N HCl were used to produce ClO_2. The generation time was decreased by a factor of 10 and the ClO_2 generated was increased by 18% (Table 1.1). Increasing the excess $NaClO_2$ from 33% to 66% resulted in an increase in the ClO_2 produced of 7.7% and 1.6% when using a theoretical generation of 3 mg/l and 6 mg/l, respectively. However, the generation time also increased by 25% and 18% using a theoretical generation of 3 mg/l and 6 mg/l. The increase in generation time could have been due to a slower release of ClO_2 gas from the beaker caused by the larger volume of reactants. An excess of 33% $NaClO_2$ was used from that point forward.

1.5.3. Optimization of Measurement Units

Several revisions were implemented to optimize the ClO_2 measurement device. Initially LEDs with epoxy lenses were chosen (360nm 5mm Round LED; The Fox Group Inc., Pointe-Claire, QC, Canada). However, due to the interaction between ClO_2 and the epoxy, the LED with the epoxy lens was replaced with a silicate glass ball LED. Sapphire windows were placed over the glass lens to prevent long-term interaction between ClO_2 and the glass LED lens. This approach also made sealing and cleaning the units easier. Cyanoacrylate glue, PVC glue, and various silicones were used when attaching metal, PVC, and plastic components together to provide an airtight seal while still allowing components of the device to be removed.

The stability of the current being supplied to the three LEDs was improved using a 24 V power supply (the photodiode measurement unit, spectroscopic measurement unit, and on/off switch indicator LED). This power supply provided sufficient voltage to

maintain the maximum set current at approximately 30.0 mA. After this change, a slow steady increase in the current was observed. The increase in the current was observed as high as 1.5 mA over the span of a couple of hours. The increase in current was prevented by cooling the resistors with a small computer cooling fan.

The two resistors used in parallel had a temperature coefficient of +350 to -450ppm/°C (KOA Speer Electronics, Inc., 2009) indicating for every million ohms used, the resistance changes up to -450 Ω for every °C the resistor changes from its initial temperature. In theory, the two resistors in parallel as well as the potentiometer could all contribute to the heating effect. However, since the potentiometer was set to allow all the current to pass through, it did not need to be considered. To determine if the difference in resistance could account for the increase in current, the two resistors in parallel (56Ω and 180Ω) were considered (calculations in Appendix J). The average initial current when the LEDs were turned on was I_i = 29.5 mA, so the voltage was determined to be 1.26V. When using a 1,000,000Ω the resistance through the resistors could change a maximum of -450Ω/°C. Since only 42.7Ω was used, the maximum change in resistance was expected to be -0.0192Ω/°C. The total temperature change required to cause a change in resistance large enough to account for the observed current drift would expected to be 109°C. It seemed improbable that the resistor would have an internal temperature change of 109°C. A 25ppm/°C, 1/4 watt, 42.2Ω resistor (IRC-TT Electronics, Corpus Christi, TX) was installed to reduce the temperature gain by the resistor. After installation of the single resistor, the current increase was limited to approximately +0.8 mA over the span of about 2-3 hours, an improvement from the two

resistors in parallel. Similar calculations (Appendix N) can be done to show the change in resistance was reduced from -2.1Ω to -1.0Ω. Because a resistance change of -1.0Ω in the high temperature coefficient resistor can be produced by an approximately 900°C increase, it was concluded that there was an additional component of the circuit causing the drift in current. However, since the effect of the drift could be eliminated by allowing the measurement unit to warm up, further modification options were not explored.

When the photodiode measurement unit had a path length of 8.2 cm between the LED and the photodiode, only 0.5V of the total 2.4V of the measurement range were being utilized. The photodiode measurement unit was modified by shortening the path length from 8.2 cm to 6.1 cm to use 0.7V of the measureable range, increasing the resolution of the device. Increased resolution is represented by an increase in the slope of the lines in figure 1.11. Because of this modification there is a greater ability to distinguish between two points in the 6.1 cm path length embodiment as compared to the 8.2 cm embodiment.

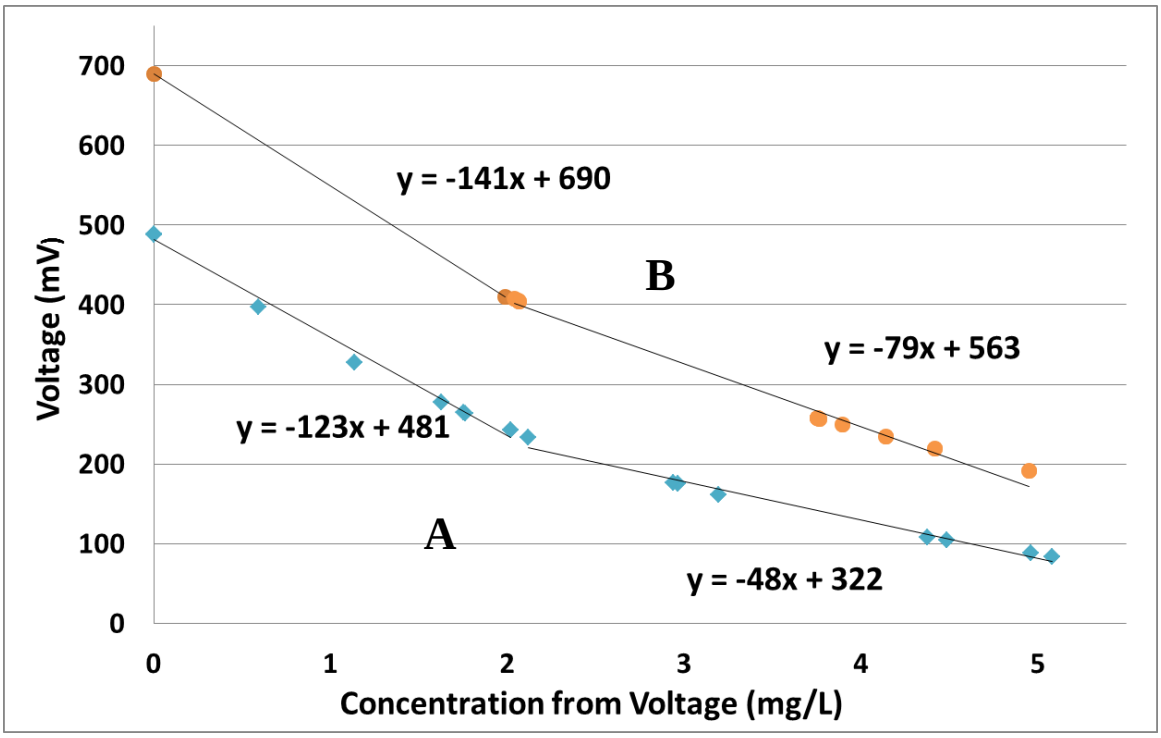

Figure 1.11. Resolution comparison between the 6.1 cm photodiode measurement unit (orange, curve B) and the 8.2 cm photodiode measurement unit (blue, curve A).

The resolution in the 6.1 cm embodiment was 13% higher between 0 mg/l and 2 mg/l, and 39% higher between 2 mg/l and 5 mg/l ClO_2 based on a comparison between the two slopes within those ranges.

1.5.4. Calibration of Photodiode Measurement Unit

Figure 1.12 shows the ClO_2 concentration in the sealed treatment chamber as a function of time, both in terms of measured voltage by the photodiode measurement unit and absorbance measured by the spectrophotometric unit.

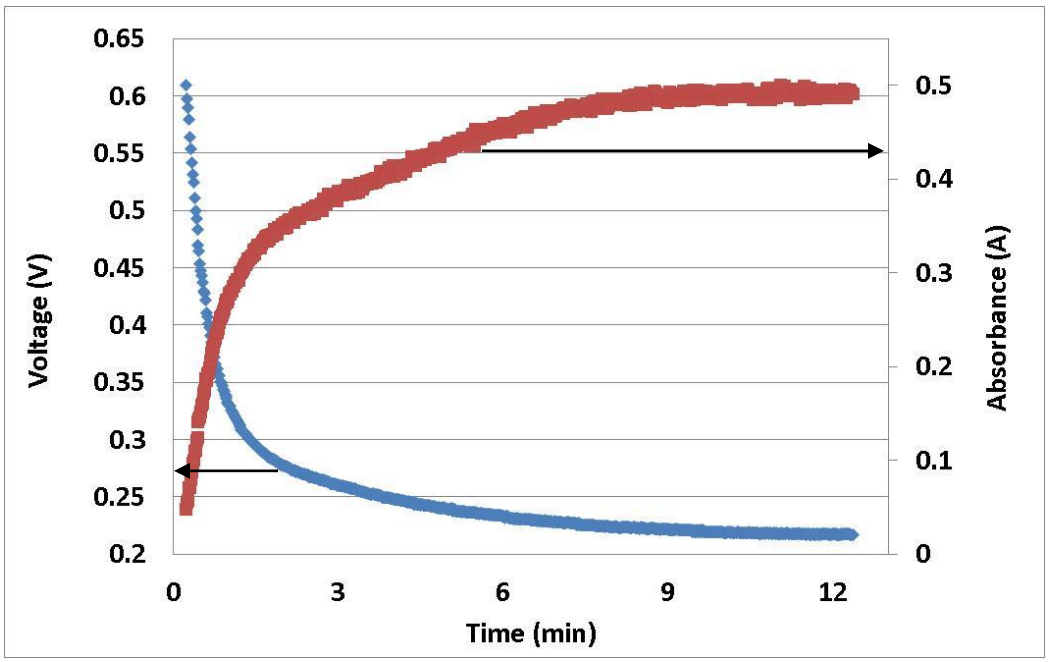

Figure 1.12. Data logged using LabVIEW from the production of approximately 4.5 mg/L ClO_2 before being converted into concentrations. Voltage (blue diamonds) and absorbance (red squares) axis are shown by arrows.

As the concentration of ClO_2 increases in the treatment chamber, more ClO_2 absorbs the UV light being emitted by the LED. As less UV light reaches the active region of the photodiode, less leakage current occurs through the photodiode, resulting in a lower voltage. Note that in figure 1.12 the voltage change begins immediately and that nearly 80% of the final ClO_2 is generated within the first 1.5 minutes. In contrast to the preliminary results shown in figure 1.8, the response of the measurement units to the increasing ClO_2 within the treatment chamber is immediate. While it took 100 minutes to reach a constant ClO_2 concentration when 7.5% $NaClO_2$ and 1 N HCl were used (figure 1.8), it took only 10 minutes using 35% $NaClO_2$ and 6N HCl. Figure 1.12 also demonstrates that the two fans within the treatment chamber provide a uniform distribution of ClO_2 inside the chamber.

Figure 1.13 shows the ClO_2 absorbance found using the photodiode measurement unit (equation 1.12) and the spectroscopic measurement unit throughout the duration of an experiment. Because the path length between the two devices were different (6.1 cm for the photodiode measurement unit and 5.95 cm for the spectrophotometric unit), absorbance values were adjusted for both measurement unit for a path length of 6.1 cm.

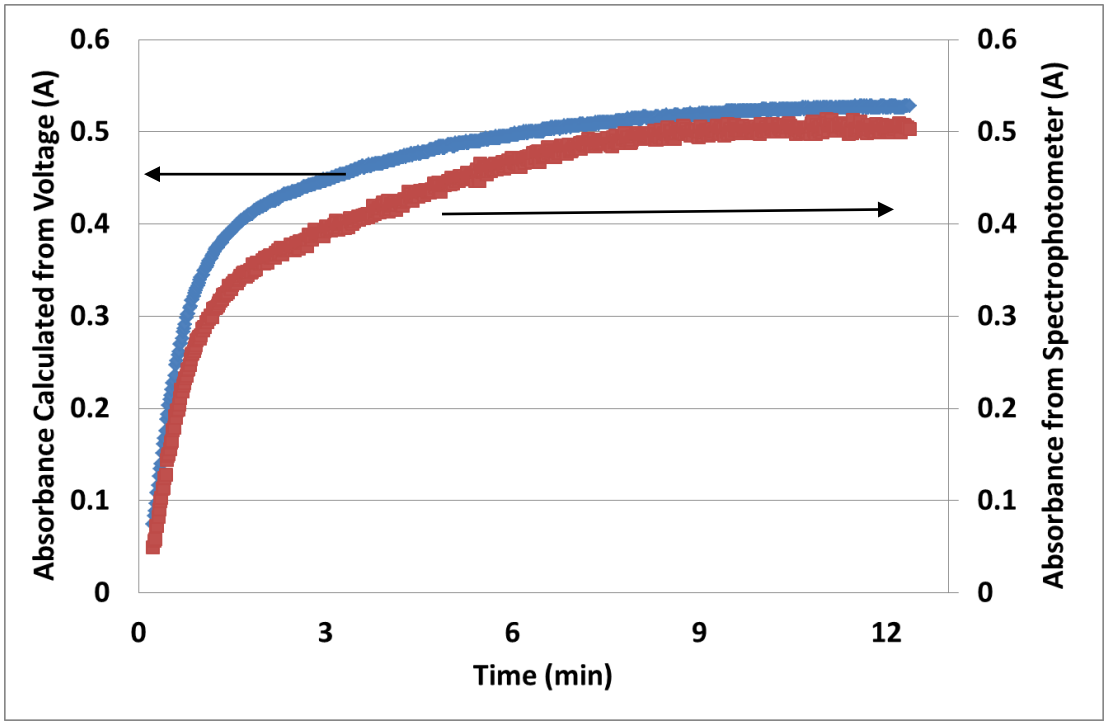

Figure 1.13. Absorbance measurements as a function of time to produce a final ClO_2 concentration of approximately 4.5 mg/l. The calculated absorbance from the photodiode measurement unit (blue diamonds) and from the spectroscopic measurement unit (red circles) for a path length of 6.1 cm.

In order to calibrate the photodiode measurement unit with respect to the spectrophotometric measurement, the relationship between the concentration measurements of the two units was investigated. It is important to note that although the

reactants ($NaClO_2$ and HCl) were mixed to obtain a theoretical ClO_2 concentration of 10 mg/l in the 44.6 L treatment chamber, the actual concentration inside the treatment chamber was approximately 4.48 mg/l. This reinforces the importance of the measurement of the concentration of ClO_2 gas being used for fresh produce treatment.

The comparison of the ClO_2 concentration calculated from the photodiode measurement unit and from the spectrophotometric measurement unit revealed a linear relationship (Figure 1.14).

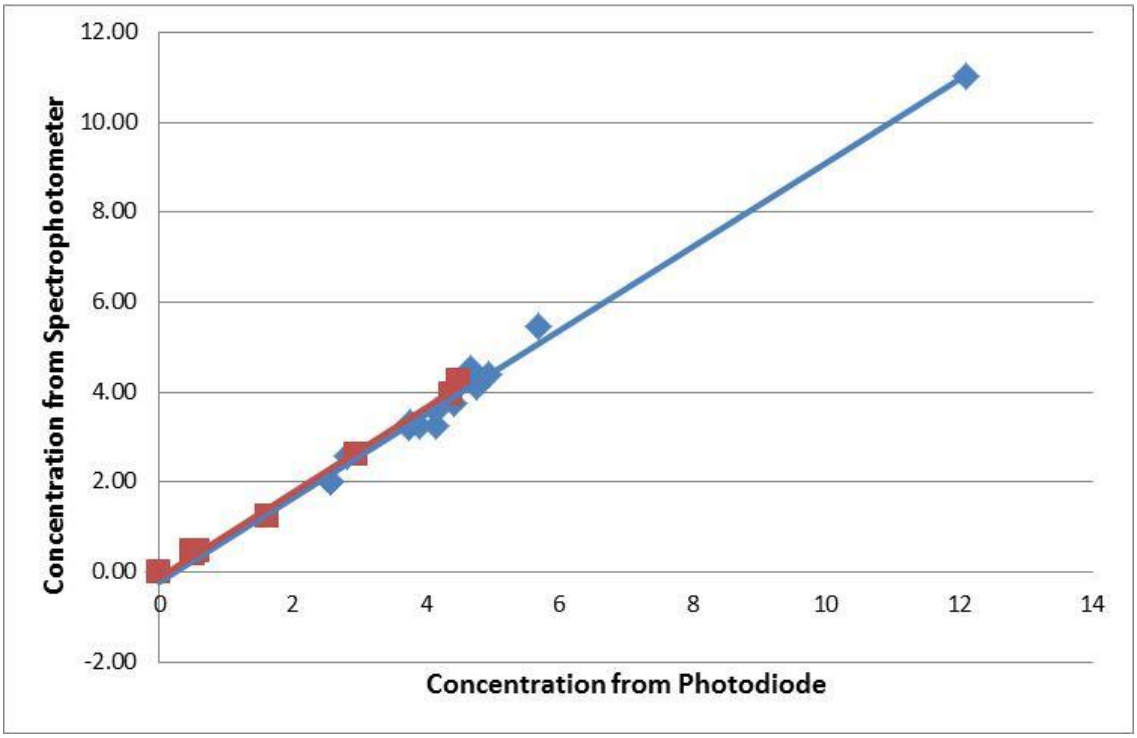

Figure 1.14. Concentration found using both photodiode measurement units. The 8.2 cm photodiode measurement unit (red squares) and the 6.1 cm photodiode measurement unit (blue diamonds) are shown.

Using the least square means Tukey test, it was found that there was no statistical difference between the equations of the data collected using the 8.2 cm and the 6.1 cm path length for the photodiode measurement unit, nor the combination of the two groups of data with a significance level of 0.05 (Appendix I). For this reason, both sets of data were used to determine the calibration equation between the two instruments. So the concentration calculated from the voltage measurement can be converted to the actual concentration using equation 1.14.

$$C_{actual} = 0.9328 * C_{measured} - 0.1810 \quad (1.14)$$

where $C_{measured}$ is the concentration calculated from the photodiode measurement unit, and C_{actual} is the adjusted concentration based on the spectroscopic measurement unit.

Differences between the photodiode and spectrophotometer measurement units can be attributed to differences between the two light intensity detection components. The responsivity curve for the photodiode is shown in figure 1.6. The LED is emitting a range of wavelengths, and the amount of current that passes through the photodiode varies by wavelength. In contrast, the spectrophotometer collected an absorbance reading for 28 different wavelengths and a polynomial fit of the data was made. The absorbance reading for the spectrophotometric measurement unit was then taken at 360 nm UV light allowing for an absorbance at a specific point rather than a range of points.

1.5.5. Precision and Resolution

The precision of the ClO_2 concentration measurement device was calculated based on the data given in Table 1.2. The precision of the instrument was defined as the

standard deviation of the distance of the points in Figure 1.14 from the equation of the line (equation 1.14) (University of Arizona, 2010). The distance was calculated by determining the difference between the measured concentration and the actual concentration and using equation 1.15.

$$a^2 + b^2 = c^2 \tag{1.15}$$

where a is the difference in adjusted concentration, b is the difference in measured concentration, and c is the distance deviated from the equation. The standard deviations of the differences were calculated and the precision was found to be ± 0.19 mg/l (Table 1.2). The precision of the photodiode measurement unit describes the maximum value that the photodiode measurement unit will differ from the true concentration as defined by the spectrophotometric measurement unit.

Table 1.2. Data used to determine precision created using the solutions to equations 1.14 and 1.15.

Measured Concnetration	Adjusted Concentration	Difference in Adjusted Concentration (a)	Difference in Measured Concentration (b)	Distance Deviated from Equation (c)
mg/L	mg/L	mg/L	mg/L	mg/L
4.67	4.52	0.38	-0.41	0.56
2.81	2.58	0.17	-0.19	0.25
3.75	3.23	-0.05	0.06	0.08
3.76	3.33	0.04	-0.04	0.05
3.75	3.28	0.00	0.00	0.00
4.14	3.24	-0.40	0.43	0.59
4.42	3.75	-0.15	0.16	0.22
3.90	3.25	-0.16	0.18	0.24
4.95	4.38	-0.01	0.02	0.02
4.73	4.27	0.08	-0.09	0.12
4.74	4.29	0.09	-0.09	0.13
4.76	4.11	-0.10	0.11	0.15
5.69	5.47	0.39	-0.42	0.57
2.57	2.00	-0.18	0.19	0.27
4.14	3.57	-0.07	0.08	0.11
2.56	1.99	-0.18	0.19	0.26
0.00	0.00	0.21	-0.23	0.31
12.10	11.02	-0.03	0.04	0.05
				STDev
				0.19

The resolution was limited by the piece of equipment, either the data acquisition board or the Ocean Optics spectrophotometer, which contained the least bits of resolution. The resolutions of both measurement units are highest at lower concentrations and lower at higher concentrations. The resolution was calculated within

the ranges from 0-2 mg/l and 2-5 mg/l by using the worst resolution within each range (2 mg/l and 5 mg/l, respectively) (calculated in Appendix K).

Based on the data acquisition board and spectrophotometer resolution, the smallest increment measureable is 0.595 mV and 6.88×10^{-5} A, respectively. The voltage value determined for the data acquisition board needed to be converted to an absorbance value so a comparison could be made between the resolution of the data acquisition board and the spectrophotometer. The $A_P = 3.78 \times 10^{-4}$ A, where A_P is the absorbance calculated from the photodiode measurement unit. Since A_P is larger than the absorbance resolution from the spectrophotometer, the data acquisition board is considered to be the component limiting the resolution of the system. Choosing the two points 2 mg/l and 5 mg/l from figure 1.11, the resolution was calculated. It is concluded that the resolution of the ClO_2 concentration measurement system is 0.04 mg/l between 0-2 mg/l and 0.05 mg/l between 2-5 mg/l (See Appendix K).

1.5.6. Treatment Chamber Testing for Kinetic Studies

Because exposure times as short as thirty seconds were used during kinetic studies, it was important to determine the length of time required for the sample chamber and treatment chamber to reach the same ClO_2 concentration. Once the concentration reached a constant value within the treatment chamber, the sample chamber was opened. The time it took to reach a constant concentration was then measured. Similarly, at the end of the exposure, the ClO_2 gas in the sample chamber needed to be purged very quickly. For this test, the photodiode measurement unit was placed inside the sealed

sample chamber and the spectroscopic measurement unit was placed inside the treatment chamber (Figure 1.15).

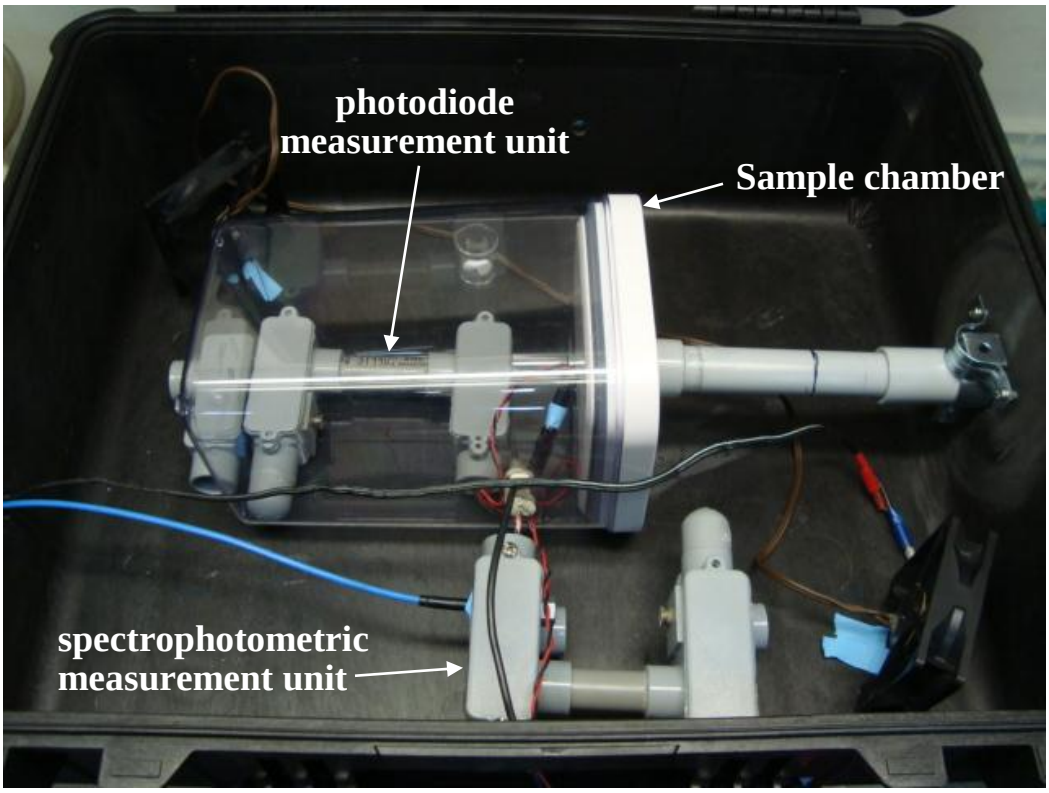

Figure 1.15. Experimental set up to determine the length of time before steady state is reached between the treatment chamber and sample chamber.

Once the ClO_2 concentration reached a constant level within the treatment chamber, the sample chamber lid was opened, exposing the samples to ClO_2 gas for the desired period of time. Two fans were located in opposite corners of the treatment chamber to facilitate a uniform distribution of ClO_2 gas within the chamber. Figure 1.16 shows the increasing ClO_2 concentration measured by the spectroscopic measurement unit, while the concentration remained constant inside the sealed sample chamber since ClO_2 was unable to penetrate into the chamber.

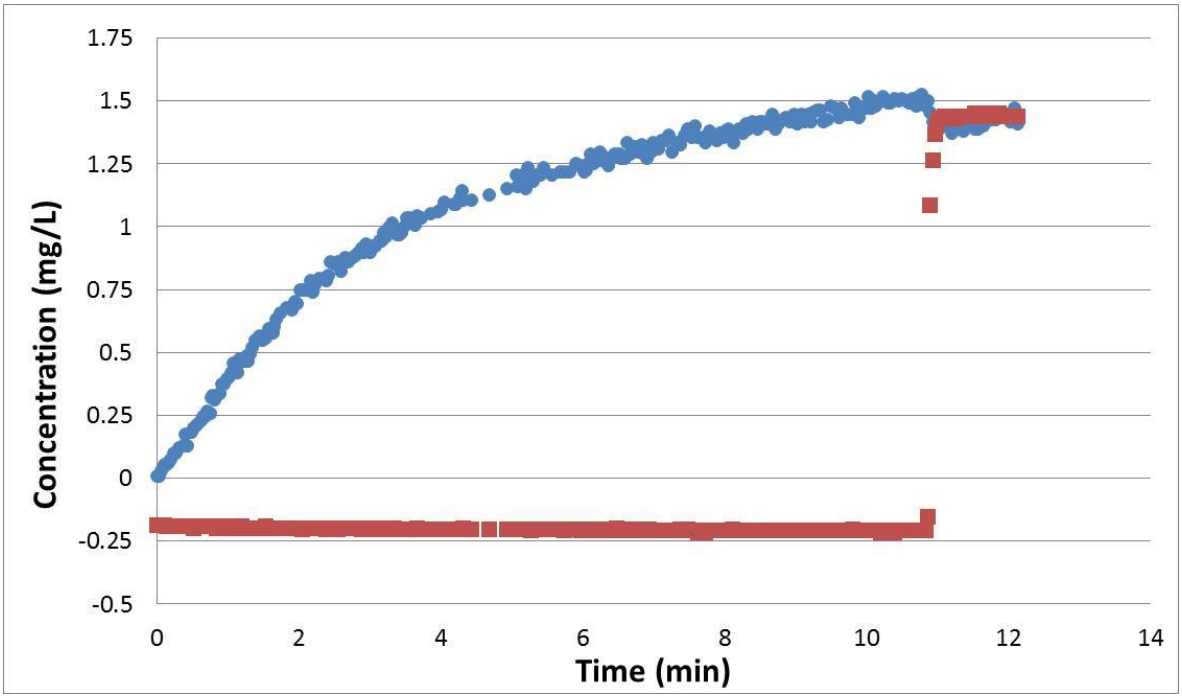

Figure 1.16. Concentration of ClO_2 gas measured by the photodiode measurement unit within the sample chamber (red squares) and that of measured by the spectroscopic measurement unit outside the sample chamber (blue circles).

When the sample chamber was opened, the concentration in the treatment chamber decreased and the concentration in the sample chamber increased to a constant ClO_2 concentration. The concentration calculated from the spectroscopic measurement unit in the treatment chamber prior to opening the sample chamber was 1.53mg/l. The volumes of the treatment chamber with the sample chamber closed and opened were approximately 39.3 L to 43.6 L, respectively. Therefore, the ClO_2 concentration was expected to reduce to 1.38 mg/l due to the volume change. The experimental data showed 1.39 mg/l to be the concentration after opening the sample chamber (Figure 1.16). Figure 1.17 shows part of the data from Figure 1.16 corresponding to the volume

change indicating that it takes six seconds for the sample chamber to reach a constant ClO_2 concentration. The six second time period is less than the smallest time duration for exposing the samples to ClO_2 gas for kinetic studies and can be used to correct the time of exposure.

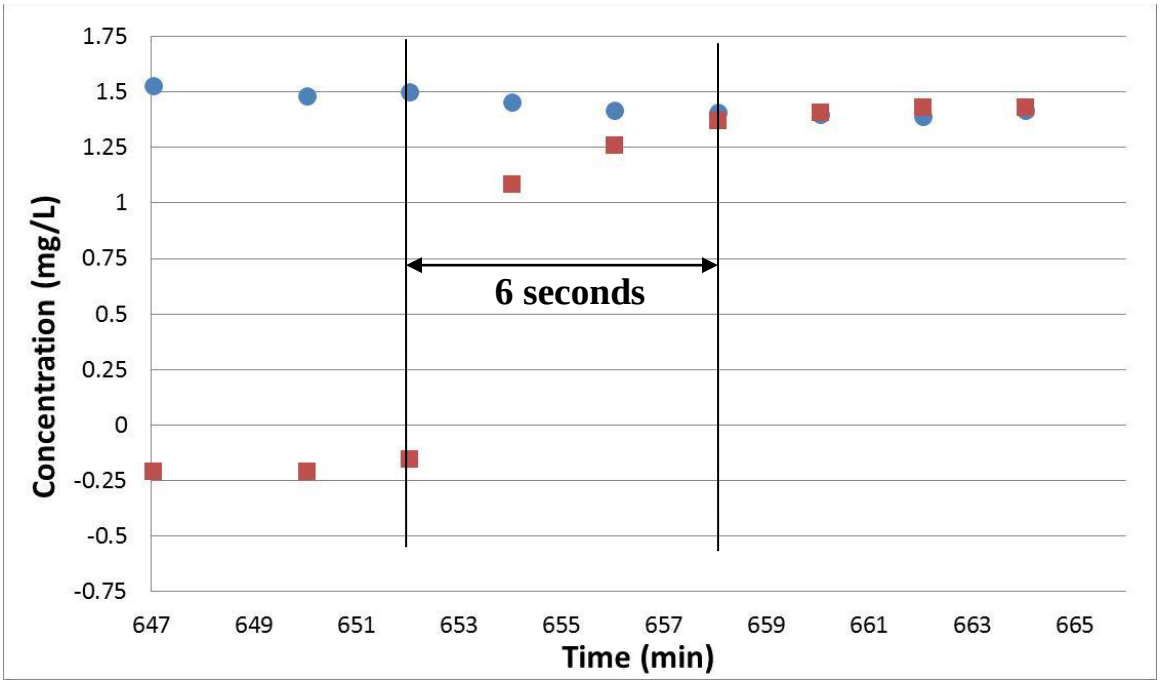

Figure 1.17. The concentration from the voltage (red squares) and spectroscopic measurement units (blue circles) in the seconds surrounding the sample chamber being opened.

After completion of the sample treatment, the ClO_2 gas in the treatment chamber needs to be purged almost immediately to obtain an accurate data during kinetics studies. Figure 1.18 shows the ClO_2 gas concentration during the purging of the gas.

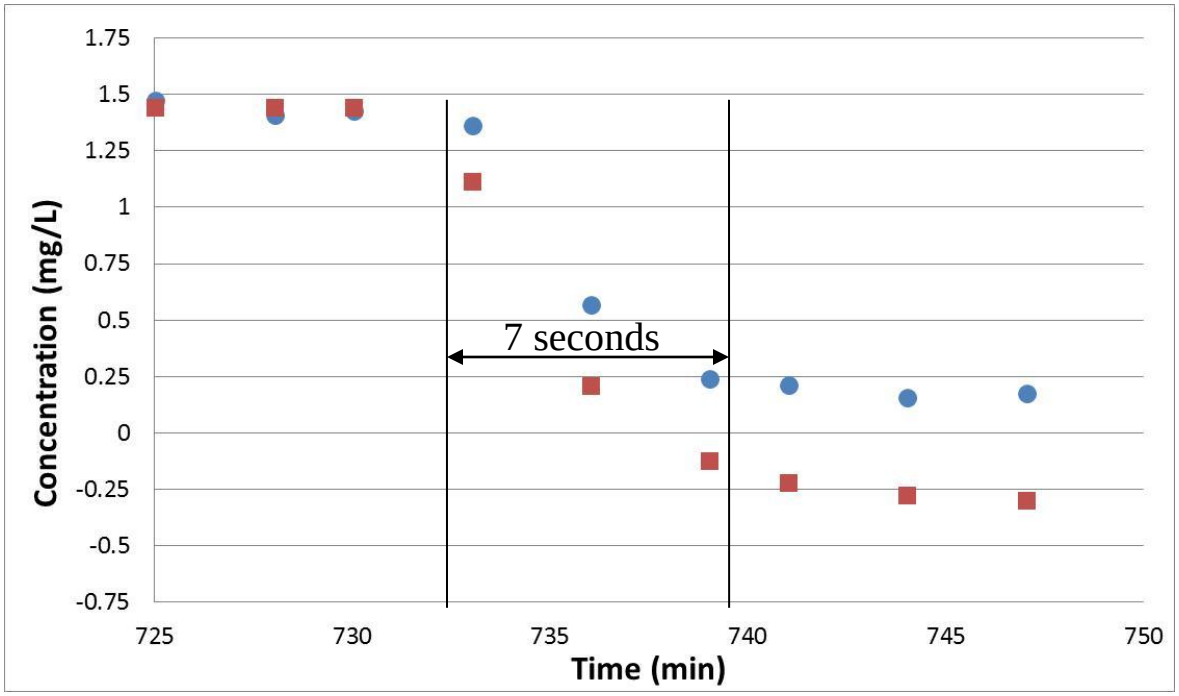

Figure 1.18. The ClO_2 concentration from the photodiode measurement unit (red squares) and spectroscopic measurement unit (blue circles) during purging of the treatment chamber.

Because the ClO_2 concentration between the two chambers equilibrates and evacuates within in a short period of time, exposure times of as short as thirty seconds can be applied during kinetics studies using this system.

1.6. Conclusions

A ClO_2 measurement unit was designed, built, and optimized to study the application of ClO_2 gas for the sanitization of fresh produce. The photodiode measurement unit was calibrated against a spectroscopic measurement unit and concentration of ClO_2 gas was corrected with a linear equation. Numerous trials validated the reproducibility of the photodiode measurement unit readings, allowing it to function

as a standalone measurement unit. The precision and resolution of the unit were ±0.19 mg/l and 0.05 mg/l, respectively, when measuring concentrations up to 5 mg/l. The cumulative parts used in the construction of this device and the accompanying circuit cost no more than $150. Throughout the optimization process the measurement unit was altered several times eventually resulting in a robust design to assure the longevity of the instrument. The sample and treatment chamber setup was successfully constructed to conduct kinetic studies.

The device measures from 0-5 mg/l with an accuracy of 0.19 mg/l and a resolution ranging from approximately 0.04-0.05 mg/l. Simple modifications could be made to the device that would allow the concentration ranges with the highest resolution to be altered (shown in Figure 4.1). Furthermore, both the voltage and spectroscopic measurement units can be controlled by the LabVIEW interface allowing the units to collect and log data in a user-friendly manner. The construction of the sample chamber and the modifications to the treatment chamber has allowed for easy setup and data collection as well as consistent treatment conditions. Chapter 2 explains the utilization of the equipment as applied to kinetic studies of microbial reduction and color change of ClO_2 treated spinach leaves.

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Chapter 2: Optimization of Chlorine Dioxide Gas Application for Spinach Sanitization: Studies on Kinetics of Microbial Reduction and Color Change

2.1. Introduction

2.3.1. Fresh Produce Safety

Due to an increased awareness of the health benefits of fresh produce, more consumers are making an effort to increase fruit and vegetable intake. In addition to health benefits, fresh produce also provides convenience to consumers. As the consumption of fruits and vegetables continues to increase, so does the threat of food related outbreaks. To increase consumer safety, improvements must be made during the harvest, transportation, cooling, processing, and retail of fresh produce. It is important that those responsible for each step are informed of, and consistently in compliance, with published FDA guidelines.

While food sanitation techniques have improved with the development of new processes, so has the number of outbreaks detected. Improved surveillance, reporting, and communication have helped the CDC to determine the outbreak source and announce recalls to the public more rapidly. However, fresh produce recalls still have limited success due to the short shelf life of fresh leafy products (15-19 days). In addition, food materials are shipped all over the world causing traceability of produce to become even more difficult. Because detection, source identification, and notification can take several weeks, it is usually too late to warn the consumer. It is estimated that the annual

economic cost in Ohio of foodborne illness; including safety programs, loss of product, and medical bills, is between $1.0 and $7.1 billion (Scharff et al., 2008).

Leafy vegetables provide additional obstacles that must be overcome to assure a safe product. Due to the irregular shape of vegetables such as spinach, lettuce, cabbage, and sprouts, pathogens may be embedded within crevices and damaged areas. In addition, leafy plants tend to have more stomata, as opposed to vegetables with less penetrable rinds (Takeuchi et al, 2000). Vacuum cooling has been proposed to lead to deeper penetration distances and larger cell concentrations of *E. coli* O157:H7 in stomata of lettuce (Li et al., 2008). Vacuum cooled lettuce contained 1.12 log CFU/g more *E. coli* than the control at room temperature when initially containing statistically similar amounts on their surface. Figure 2.1 shows bacteria highly concentrated inside the stomata of leafy greens and less concentrated on the surface.

Figure 2.1. *E. coli* shown deep within produce stomata (Gomes et al., 2010).

E. coli has been one of the major microorganisms associated with fresh produce contamination, causing sickness and death in some of the most high profile outbreaks. In

addition to *E. coli*, *Listeria monocytogenes* (*L. monocytogenes*) and *Salmonella* are among the most prevalent pathogenic microorganisms associated with fresh produce (Beuchat, 1995).

2.3.2. Critical Control Points in the Fresh Produce Supply Line

There are many steps within the fresh produce production chain that can cause contamination to occur or spread. Figure 2.2 shows the typical steps and their duration within fresh produce processing.

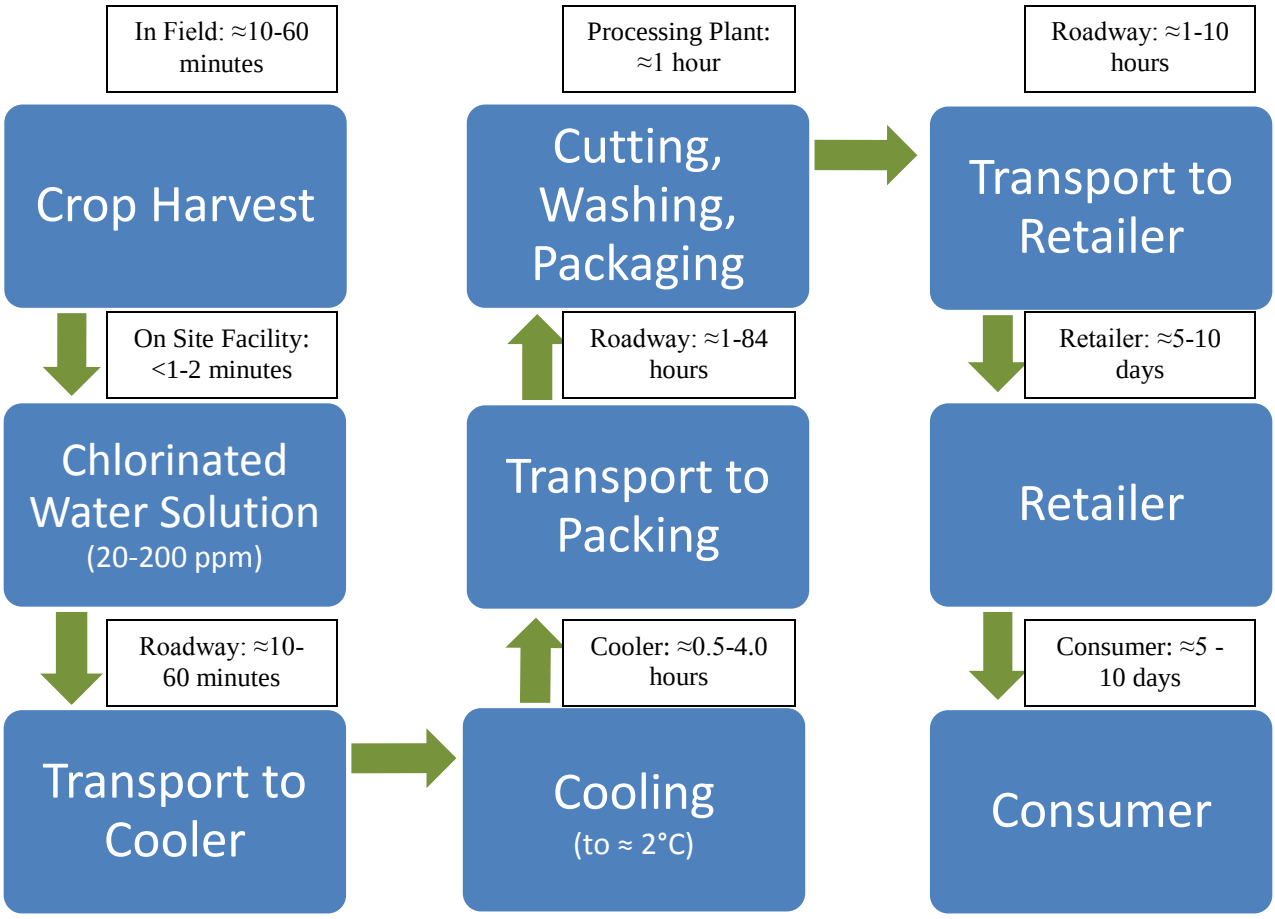

Figure 2.2. Current sanitation process for fresh green leafy produce including the approximate time elapsed at each unit.

In the field, crops are subjected to an uncontrolled environment. Microorganisms from the soil or animals have the potential to contaminate the produce while growing in the field. Microbial populations were found to be 5.7 log CFU/g, 5.2 log CFU/g, 6.1 log CFU/g, and 6.4 log CFU/g on cilantro, parsley, mustard greens, and cantaloupe, respectively, just after being harvested (Johnston et al., 2005). Since these large microbial loads are not avoidable in an open field environment, sanitation practices have to be even more effective. Each processing step has the potential to contribute additional

bacteria to the fresh produce. Johnston et al., (2005) reported after washing, rinsing, and boxing, aerobic microbial populations had actually increased to 6.7 log CFU/g, 6.0 log CFU/g, 6.3 log CFU/g, and 7.0 CFU/g, on cilantro, parsley, mustard greens, and cantaloupe, respectively.

When produce is harvested in the field, there are additional contamination possibilities. Using a blade, the crop is removed from the ground and placed in a storage bin, preferably with a reflective cover to reduce produce heat gain (Suslow, 2000). If the produce is removed from the field by human worker, microbes and viruses can easily be transferred from the worker to the produce. Because damaged areas are more difficult to sanitize, extra care must be taken to avoid excessive damage to the product during harvesting (Takeuchi et al., 2000). Produce sanitation procedures should be conducted as quickly as possible to prevent the further accumulation of biofilm on the vegetables surface. After harvest, chlorinated water is applied by either submerging the product in, or spraying the product with a chlorine concentration of 20-200 ppm. Submersion for one minute commonly achieves a microbial log reduction of approximately 1.3-1.7 CFU/g (Beuchat et al., 1998). Although chlorinated water washes can reduce the microbial load on produce, it is mainly used to prevent cross contamination from one leafy green to another while removing organic material from the crop.

After the organic matter is rinsed off the produce, it is cooled from field temperature to an optimal storage temperature (0 °C for leafy greens) very rapidly to preserve quality (Sargent et al., 2007). The four most common cooling techniques for leafy vegetables are ice-packing, forced air cooling, hydro-cooling, and hydrovac cooling

(Sargent et al., 2000). Ice-packing involves filling the spaces between the produce with crushed ice in an ice to product ratio of approximately 1:3. Forced air cooling involves chilled air flowing over specially stacked bins of produce in a refrigerated room. Hydro-cooling involves placing the produce on a conveyor belt that is doused with chilled water, cooling the produce in approximately twenty minutes. Hydrovac cooling involves cooling vegetables under a vacuum while spraying the produce with water to reduce water loss and shorten cooling times (Sargent et al., 2000). Hydrovac cooling is extremely effective in preserving food quality because of rapid cooling rates of less than 30 minutes (McDonald and Sun, 2000). All water being used throughout the process should contain at least 2-20 ppm chlorine to reduce cross contamination (McGlynn, 2004).

The produce is then transported on refrigerated trucks. The transportation time could take 3-5 days including travel from the field to the cooler, to the processing plant, to the retailers. At the processing plant, additional processing steps are applied, some of which include cutting, washing, mixing, and packaging. Once the produce arrives at the retailer, temperature fluctuations are minimized using industrial refrigeration systems to keep the fresh produce at a reduced temperature (0°C for leafy greens) until the product is purchased by the consumer (Robinson et al., 1975). Temperature changes could cause moisture loss, further reducing the shelf life of fresh produce. Since the shelf life of fresh leafy green vegetables is short, emphasis should be placed on proper storage conditions throughout the shelf life of the product.

2.3.3. Sanitation Methods for Fresh Produce

2.3.3.1. Liquid Sanitizers

There are several liquid sanitizers that could be used to eliminate microorganisms, including chlorine dioxide, ozone, peroxyacetic acid, and chlorinated water. Chlorinated water is the most frequently used in industry. It is the least expensive of all the Environmental Protection Agency (EPA) approved sanitizers for fresh produce because of the low cost of the active ingredient, application equipment, and operator cost. Chlorinated water can be generated several ways (Table 2.1).

Table 2.1. Popular precursors to chlorinated water and their relative cost and safety attributes (Shah and Qureshi, 2008).

Disinfectant	Purchasable Concentrations	Purchasable Form	Cost	Safety
Chlorine Gas	100% free active chlorine	high-pressure liquid	high capital, low operation	threat of gas leak or explosion
Calcium Hypochlorite	65 or 68 % free active chlorine	tablet or granulated powder	intermediate capital, intermediate operation	more hazardous due to higher percent chlorine
Sodium Hypochlorite	5-15 % free active chlorine	water-based formulation	low capital, high operation	low level safety concerns

A long channel is filled with the liquid sanitizer and produce is passed from one end to the other allowing for 1-2 min of surface contact (Beuchat, 1998). Spray application of chlorinated water can occur during hydro-cooling, in which case the produce is placed on a conveyor belt being sprayed by chlorinated water, simultaneously cooling and sanitizing the produce.

2.3.3.1.1. Advantages of Liquid Sanitizers

Liquid sanitizers provide processors with a well-established method of disinfecting produce. The technology needed to utilize liquid sanitation methods is well understood within the fresh produce industry. Although some gaseous sanitizers, such as ozone, are permitted for direct food use, acceptance of these alternative sanitizers has been slow (Suslow, 2004). Liquid sanitizers also have the potential to be safer for those working with the sanitizer. If calcium hypochlorite or sodium hypochlorite is used properly to generate chlorinated water, then there are no hazardous gases being used in the process. Inhalation of Cl_2 or ClO_2 gas can be harmful to humans above 1 ppm or 0.1 ppm, respectively, according to the OSHA permissible exposure limit (PEL) of (OSHA(a), 1996 and OSHA(b), 1996). The properties of a liquid makes liquid sanitation possible during many steps in the production process, while gaseous sanitizers must be used in sealed containers to prevent the sanitizer from escaping. For these reasons liquid sanitizers are predominantly used in industry.

2.3.3.1.2. Disadvantages of Liquid Sanitizers

Liquid sanitizers have a number of qualities that make them an inadequate sanitizer for most fresh fruits and vegetables. The effectiveness of liquid sanitizers is reduced by the buildup of organic material, lower diffusivity, reduction in penetration depth, inability to diffuse through biofilms, and the presence of air bubbles. Since fresh produce is collected from an open field environment, a large amount of organic material

is often transported to the production facility along with the produce. Currently, the primary sanitation step for produce occurs after, or even during, the initial rinsing step. When organic material builds up in wash water, the pH of the chlorinated water begins to rise. Three compounds may exist in chlorinated water depending on the pH (Table 2.2). Since the effectiveness of chlorinated water is directly correlated to the level of hydrochlorous acid (HOCl) present in the solution, improper monitoring of pH can yield an unsafe product (McGlynn, 2004). The pH of chlorinated water should never get low enough to damage sanitation equipment and never high enough to become an ineffective sanitizer.

Table 2.2. Percentage of three compounds observed in chlorinated water at different pH levels.

pH	% Cl_2	% HOCl	% OCl^-	Chlorinated Water Description
2	30	70	0	Dangerous Cl_2 levels, too corrosive for equipment and food products
3	5	95	0	
4	0	100	0	Too corrosive for equipment and food products
5	0	100	0	
6	0	97	3	
6.5	0	90	10	Ideal range
7	0	75	25	
7.5	0	50	50	
8	0	35	65	HOCl level too low for proper sanitation of food products
9	0	2	98	
10	0	0	100	

The effectiveness of a sanitizer also depends on its ability to reach the bacteria on the produce. The time interval required for the sanitizer to come in contact with the

bacteria should be within the application time of the sanitizer. Therefore, the consideration of sanitizer diffusivity is highly relevant in the process of choosing a sanitizer. The approximate diffusivity of gas in gas is 0.1 cm^2/s and liquid in liquid is 10^{-5} cm^2/s (Middleman, 1998), although temperature, pressure, and concentration gradient all affect the diffusivity. The relatively low diffusivity of liquid sanitizers is a major contributor to its inability to successfully sanitizing fresh produce. A simple example, using the relationship between diffusivity and distance traveled (equation 2.1), can illustrate the major drawback of liquid sanitizers.

$$C = \frac{M/A}{\sqrt{4Dt}} * e^{\frac{-y^2}{4Dt}} \tag{2.1}$$

where M is the mass of the sanitizing agent at a point at the opening of the pore, A is the area of the pore opening, y is the mean squared displacement in the y direction, D is the diffusivity of the sanitizing agent through the medium (air or water), and t is the time required assuming there is no sanitizer initially present in the pore.

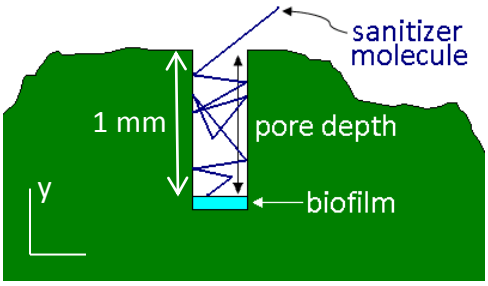

Figure 2.3. Penetration into a pore on a lettuce leaf by a single molecule of gaseous sanitizer.

If the pore is filled with liquid, and has a depth of 1mm, and an initial concentration of 5 mg/L it will take 3,300 seconds to reach a final mass of 1.0 mg at the bottom of the pore. Produce is exposed to the liquid sanitizer for 1-2 minutes (Beuchat, 1998), preventing the sanitizer from reaching the bacterium. If the same example calculation is done using the diffusivity of a gaseous sanitizer, 1.0 mg of ClO_2 will reach the bottom of the pore in 0.33 seconds. This assumes that there is not already liquid inside of the pore and that the gas is traveling through air. The sanitizer exposure time would be substantially longer than this showing that a pore 1mm in depth would be penetrated by a gaseous sanitizer.

Given enough time to settle on a products surface, many bacterial species will form biofilms (Poulsen, 1999). The most common types of microbes that form biofilms are *Pseudomonas, Enterobacter, Staphylococcus, Flavobacterium, Alcaligenes,* and *Bacillus* (Mattila-Sandholm et al., 1992). Biofilm growth can begin from the moment a biofilm-producing microorganism becomes sessile, or physically attaches to the surface of the produce. It is critical that sanitation occurs as quickly as possible, before biofilm has had time to accumulate on the surface of the produce. The effective diffusivity of a gas through a biofilm ranges from 0.2-0.8 times that of the diffusivity of water (the average is $0.4D_{water} = D_{biofilm}$), depending on the cell density and extracellular polymers (Stewart, 2003). Since the diffusivity of a gas through water is approximately 1.0×10^{-5} cm^2/s (Middleman, 1998), and the diffusivity through a biofilm is approximately 4.0×10^{-6} cm^2/s, given a biofilm thickness of 30 μm (1μm/cell, 30 cells thick), the penetration time will be 2.25 seconds. Even if the biofilm was at the bottom of the 1mm pore it would only take about 2.35 seconds to penetrate the biofilm. Although this example

assumes that the liquid carrying the sanitizing agent was able to overcome the surface tension at the opening of the 1 mm pore, it illustrates the clear difference between liquid and gaseous sanitizer penetration.

Pores, stomata, and damaged areas along the surface of many fruits and vegetables allow for deeper penetration of pathogens into the product. In some cases, openings into the product are so small that liquid sanitizers, due to the surface tension of water, cannot freely flow inside. Surface tension causes the sanitizing liquid to have a stronger cohesion with itself than it does with the organic material being sanitized. For this reason, even with the aid of a surfactant, the sanitizing liquid may not be able to enter small pores (Beuchat and Ryu, 1997). Since the average bacterium and virus are 1μm and 0.1μm, respectively, the sanitizer used has to be able to access openings that are equally small (Prescott et al., 2010).

Finally, a liquid sanitizer may not contact the entire surface of the produce due to the formation of air bubbles along the surface (figure 2.4). Air bubbles form a space which liquid sanitizers cannot penetrate lowering exposure time at that point. Also, when submerging produce in a sanitizing solution, any portion of the produce that surfaces will no longer be in contact with the sanitizing solution, lowering its effectiveness.

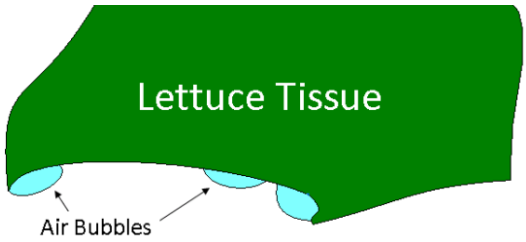

Figure 2.4. Air bubble accumulation on the bottom surface of lettuce, impeding liquid sanitizers from contacting portions of the produce.

2.3.3.2. Gaseous Sanitizers

Chlorinated water in submersion channels is widely used in the United States. While submersion channels are highly effective at removing surface debris, the removal of microorganisms requires a different approach (Sapers, 2001). A number of studies have shown that gaseous sanitizers are effective in reducing the bacterial population on produce (Table 2.3). However, a lack of engineering advances in application and safety have restricted their adoption (Guzel-Seydim et al., 2004). Gaseous sanitizers have the potential to be applied at a point where the product is enclosed in a sealed container. Studies have been conducted showing gaseous ozone application is possible during vacuum cooling, transportation to the processor, and short term storage (Vurma et al., 2009). Extended exposure time of gaseous sanitizers to green leafy vegetables could allow the use of lower concentrations, reducing the damage high concentration sanitation can cause.

Table 2.3. Various examples of ClO_2 gas sanitation of fresh produce.

Food Material	Bacteria	Inoculation Method	Gas Produced (mg/L)	Exposure Time (min)	Relative Humidity	Temp (ºC)	Log Reduction	Source
Apple calyx	E. coli O157:H7	spot, 2-3 hr. delay	10	7.2	90-95%	21	2.9	Du, J.
			20	7.2			3.8	
			30	7.2			6.5	
			10	18			3.8	
Blueberries	E. *coli* O157:H7 L. *monocytogenes* *Salmonella*	sprinkle, ≈ 24 hr. delay	4	12 hr.	99.9%	22	4.25	Popa et al., 2007
Tomatoes	*Salmonella enterica* spp.	spot, 1 hr. delay	2	2.5	90-95%	25	2.41	Trinetta et al., 2010
			5	2.5			2.43	
			8	2.5			3.77	
			10	2.5			4.87	
Injured Peppers	L. *monocytogenes*	spot, 2 hr. delay	0.3	10	90-95%	20	1.88	Han et al., 2001
			3	10			3.60	
Uninjured Peppers			0.3	10			3.05	
			3	10			7.39	
Blueberries	*Salmonella*	spot, 24 hr. delay	4.1	30	75-90%	23	2.95	Sy et al., 2005
Strawberries							2.32	
Raspberries							0.52	

2.3.3.2.1. Applications of Gas Sanitizers

Effectiveness of chlorine dioxide, ozone, and allyl isothiocyanate gas, along with a few vapors (hydrogen peroxide and acetic acid vapor), has been tested on food materials (Linton et al., 2005). The most heavily tested gaseous sanitizers to this point have been ozone and ClO_2. Ozone is well known to be effective in its aqueous form to treat drinking water (Rice et al., 1981). Bubbling gaseous ozone through liquid containing food materials has been shown to reduce aerobic bacteria by 1.9 log CFU/g on lettuce (Kim et al., 1999) and 3-4 log CFU/g on black peppercorn (Zhao and Cranston, 1995). An even larger bacteria log reduction was reported using the gaseous form of ozone directly on food material with 7.35 log CFU/g reduction of *E. coli* O157:H7 after 25 min of 8.0mg/l exposure on green peppers (Han et al., 2002) and a 6 log CFU/g reduction of four different aerobic bacteria after 60 min of 6.7mg/l exposure on black pepper (Zhao and Cranston, 1995). Vurma et al. (2009) reported 1.8 log CFU/g reduction during vacuum cooling of spinach at 10 psig, 30 minutes of exposure, and 0.20 mg/l ozone, without any apparent damage to the leaves.

The effectiveness of ClO_2 is dependent on the atmospheric conditions it was generated in. Gas concentration and exposure time, as well as, relative humidity and temperature of the environment all play a role. Han et al. (2001) showed that the log reduction of bacteria increases with increasing temperature and relative humidity at a constant ClO_2 concentration. Relative humidity especially, appeared to have a large effect on bacterial reduction. Han et al. (2001) found that there was nearly a 3 log

increase in microbial reduction on green peppers when relative humidity was increased from 55% to 95% at 0.5 mg/l ClO_2 concentration.

2.3.3.2.2. Chlorine Dioxide Application for Fresh Produce Sanitation

The EPA Scientific Advisory Panel suggests a 5 log pathogen reduction when processing juice concentrates (FDA, 2004), and the National Advisory Committee on Microbiological Criteria for Food suggests a 5 log pathogen reduction of bean sprout seeds prior to planting (FDA, 1999). However, a similar suggested minimum performance standard is not defined for fresh produce. In each of the samples shown in table 2.3, visible damage was not detected immediately after treatment. It has even been shown that there were no significant change ($p<0.05$) in color of strawberries after a 1-week storage time after being exposed to 3.0 mg/l for 10 minutes (Han et al., 2004), and green peppers after a 4-week storage period after a 0.6 mg/l exposure for 10 minutes (Han et al., 2003).

In contrast, Table 2.4 shows some exposures of leafy green vegetables that were less successful.

Table 2.4. Various examples of ClO_2 gas sanitation of leafy green vegetables.

Food Material	Bacteria	Inoculation Method	Gas Produced (mg/L)	Exposure Time (min)	Relative Humidity	Temp (°C)	Log Reduction	Appearance Testing Method	Appearance	Source
iceberg	E. *coli* O157:H7	spot, 22 hr. delay	1.4	6.4	62-98%	22±1	0.64	visual inspection	noticalbe damage	Sy et al., 2005
			2.7	12.3			0.72			
			4.1	20.5			1.57			
lettuce	E. *coli* O157:H7	spot, 30 min. delay	0.215	30	not reported	22±2	3.4	visual inspection	no difference	Lee et al., 2004
			0.335	60			4.4			
			0.435	180			6.9			
iceberg	E. *coli* O157:H7	spot, 1 hr. delay	0.5	10	90-95%	22	1.39	colorimeter/ visual inspection	substantial damage	Mahmoud et al.,2007
			1				2.94			
			1.5				3.03			
			3				3.13			
			5				3.44			
romaine	E. *coli* O157:H7	sprinkle, 24 hr. delay	0.5	5	80%	22	0.73	none	none	Singh et al., 2002
			0.5	10			1.17			
			0.5	15			1.25			
			0.75	5			0.81			
			0.75	10			1.67			
			0.75	15			1.9			
			1	5			1.04			
			1	10			1.91			
			1	15			2.21			
Spinach	E. coli O157:H7	sprinkle, 2 hr. delay	1.2	30	not reported	22	0.7	visual inspection	no difference	Neal et al., 2012
			2.1	60			0.7			

Not only were lower reductions of microorganisms observed, but there was also noticeable damage to several of these samples. Lettuce has been seen to be one of the more robust leafy greens included in the category, however, exposure to ClO_2 gas for extended periods still causes bleaching. The bleaching of leafy green vegetables has been reported in several studies (Mahmoud et al., 2008 and Keskinen et al., 2009). Even the strawberry cap leaves sustained substantial damage from chlorine dioxide treatments (Han et al., 2004). Another noteworthy observation that can be taken from the literature was the seemingly conflicting data collected in the leafy green samples. Lee et al. (2004) reports an *E. coli* O157:H7 reduction of 3.4 log CFU/g with 0.215 mg/l ClO_2, while Neal et al. (2012) reports only a 0.7 log CFU/g reduction with 1.2 mg/l, both for 30 minutes exposure time. Sy et al. (2005) and Mahmoud et al. (2007) also had similar treatments yielding much different results. Differences in procedure and source of the produce may account for some of these differences; however, further work was needed with leafy greens to determine possible sources of variability.

ClO_2, Cl_2, chlorite (ClO_2^-), and chlorate (ClO_3^-) residuals left on treated samples were investigated (Han et al., 2004). The early exploration into the post treatment safety of fresh produce was promising showing little or no residue shortly after treatment. Strawberries treated with ClO_2 at a concentration of 3.0 mg/l for 10 minutes contained 0.19 ± 0.33 mg ClO_2/kg and 1.17 ± 2.02 mg Cl_2/kg immediately after treatment, but no residual ClO_2 was detected after one week, and Cl_2 was reduced to 0.07 ± 0.12 mg/kg (Han et al., 2004). Shrimp and sea scallops were tested for ClO_2^- and ClO_3^- residuals (Kim et al., 1999). At 34.9 ppm aqueous ClO_2, 4.71 mg/kg ClO_3^- was found on shrimp,

while 9.30 mg/kg ClO_3^- was found at 15.9 ppm aqueous ClO_2 on sea scallops. No ClO_2^- was found on either sample. Because ClO_3^- is converted to chloride during cooking, the concentration levels were deemed not to be a health concern (Kim et al., 1999). More work needs to be done to determine what lengths of time would be sufficient to guarantee residuals low enough to be safe for the consumer.

2.3.3.2.3. Chlorine Dioxide Properties

Chlorine dioxide is a yellowish orange gas at standard temperature and pressure. Chlorine dioxide is an oxidizing agent with approximately 2.5 times the oxidation capacity of chlorine (Benarde, 1967). It is stable in air up to 10% (v/v) and it has a solubility of 2.20×10^{-2} g/cm^3 in water (Young, 1983). ClO_2 does not hydrolyze in water but remains in the solution as a dissolved gas. As aqueous ClO_2 reacts with organic material, it breaks down into chlorite and chlorate. UV light and high temperatures cause the decomposition of ClO_2 into chlorate and chlorite (American Water Works Association (AWWA), 1990). ClO_2 gas decomposes into chlorine gas and oxygen when held at high temperatures, exposed to UV light or organic material, and when subjected to sudden pressure fluctuations (Linton et al., 2005). ClO_2 can be explosive at concentrations over 10% (v/v), and therefore cannot be transported as a gas. The disinfection mechanisms that occur with chlorine dioxide treatment are twofold (Olivieri et al., 1985). ClO_2 reacts readily with the amino acids cysteine, methionine, tryptophan, and tyrosine causing damage to many proteins in the outer membrane, disrupting the ionic gradient, causing an increase in cell membrane permeability (Aieta and Berg,

1986). ClO_2 also, further damages proteins and enzymes within the cell that are necessary for protein synthesis (USDA, 2002). More specifically, bacterial reductions of greater than 5 log CFU/g were observed on cantaloupe (Salmonella) (Mahmoud et al., 2008) and on apples (*E. coli* O157:H7) (Du et al., 2003).

2.2. Objectives

The purpose of this study was to determine what ClO_2 concentrations and time of exposure would provide the maximum microbial reduction while minimizing the color change of spinach. Experimentation to test the effectiveness of ClO_2 as a sanitizer began with the analysis of color change caused by ClO_2 gas on spinach leaves. Areas of interest were defined and explored further by testing the microbial reduction attainable with varying concentration-exposure time combinations. ClO_2 gas has been shown to be effective in eliminating bacterial spores on paper, plastic, epoxy-coated stainless steel, and wood (Han et al., 2003). ClO_2 gas was shown to be an effective decontamination compound, not only on inert surfaces, but also on a variety of food surfaces.

1. To optimize ClO_2 gas concentration and exposure time combinations to maximize microbial reduction while minimizing color change of spinach leaves.
2. Determine the relationship between the kinetics of microbial reduction and the kinetics of color degradation of spinach sanitized using ClO_2 gas.

2.4. Materials and Methods

2.4.1. Materials

2.4.1.1. Media

For all cell growth, 3% tryptic soy broth (TSB) by mass (EMD Chemicals, Inc., Darmstadt, Germany) was used. Nalidixic acid (169900250; Acros Organics, Fairlawn, NJ, USA) and acriflavine (190675; MP Biomedics LLC, Solon, OH, USA) were used to prepare growth media for *E. coli* resistant to these chemicals (Lee et al., 2011). All dilutions were made using 0.1% peptone water. Peptone used for the ten-fold dilutions after ClO_2 exposure required the addition of neutralizers (Lalla et al., 2005). Neutralizer peptone contained 0.1% peptone (J636; Amresco LLC, Solon, OH, USA), 0.5% Tween 80 (278632500; Acros Organics, Fairlawn, NJ, USA), and 0.1% sodium thiosulfate (S446; Thermo Fisher Scientific, Pittsburgh, PA, USA).

2.4.1.2. Spinach

All spinach seeds (Double Choice Hybrid) were purchased from Burpee Gardens (Warminster, PA, USA) and grown in a greenhouse at the Ohio State University under closely regulated conditions. Greenhouse temperature was held between 20-27°C, spinach was watered once a day, and shade was provided to lengthen the time between germination and flowering. As many as 650 plants were cultivated at a time providing a sufficient selection of spinach to prepare uniform samples. Leaves were harvested after 47-69 days, depending on the rate of growth for that particular lot, the morning of the experiment. Spinach plants were grown without pesticides or growth restrictors and

watered once a day. Spinach was grown under shade cloth in an approximate temperature of 20-27°C. Plants were hand planted and transplanted and were grown approximately 2-3 inches apart until harvest.

2.4.1.3. E. coli K-12

E. coli K-12 was used to inoculate the spinach leaves in all microbial studies. The specific strain chosen was obtained from Ohio State University Food Science Department (Columbus, OH, USA), and contained a plasmid providing a resistance to nalidixic acid. Acriflavine and nalidixic acid were used in growth media to eliminate gram (+) and nonresistant gram (-) bacteria, respectively. *E. coli* K-12 was grown on agar slants at 37°C then stored at 4°C until needed.

2.4.2. Methods

2.4.2.1. Spinach Selection and Handling

Spinach leaves of similar size and age were harvested from plants in the greenhouse using gloves and sterile sheers. Leaves were chosen only if they were free of visible injury or defect. All samples consisted of five spinach leaves of approximately the same total mass. As the study progressed, it became clear that the upper and lower axillary leaves differed in both color and shape. For this reason, extra care was taken to choose groups of leaves in a way which would reduce variability between samples. Every sample of five leaves included three leaves from the higher axillary buds (upper leaves) and two from the lower axillary buds (lower leaves). The color parameters of all

the samples were measured prior to inoculation and ClO_2 treatment. They were then stored inside a rigid container (LBH4711-1; Plastic Container City, New York, NY, USA) within a polyethylene bag (Ziploc; S. C. Johnson & Son, Inc., Racine, WI, USA) in a refrigerator at 4°C prior to the ClO_2 treatment. Samples were then randomly assigned either, 1) inoculation and ClO_2 exposure, 2) inoculation without ClO_2 exposure, 3) no inoculation and ClO_2 exposure (for color studies), or 4) no inoculation and no ClO_2 exposure (to see natural micro flora initially present). Before each treatment the 55 leaves were weighed and their initial mass recorded. The 55 leaves accounted for the 11 samples, and the inoculum used to inoculate the spinach accounted for one additional set of plates (see table 2.5).

Table 2.5. Typical sample and media setup for microbial studies.

Treatment		Media			Plates
Inoculation	ClO_2 Treatment	Number of each Sample	Chemical Plates Needed	Plates with no Chemicals Needed	Dilutions (10^x)
Initial Peptone Inocula		1	6	0	-4-5-6
Yes	Yes	4	28	0	0,-1,-2
Yes	No	4	24	0	-2,-3,-4
No	Yes	2	0	0	no dilutions
No	No	1	2	2	-1
Total		12	60	2	22 pep tubes

2.4.2.2. Spinach Color Measurements

2.4.2.2.1. Spinach Quantitative Color Measurement

Color is an important quality of fresh leafy greens. Therefore, the color of the spinach samples was measured before and after exposure to ClO_2 gas. Controls were measured in each study to determine the color change in leaves that were not exposed to ClO_2 gas. The color of spinach leaves was measured using a colorimeter (ColorQuest XE; HunterLab Inc., Reston, Virginia, USA) before and after treatment, as well as intervals throughout storage of the spinach. Measurements were made at three locations on both the upper (adaxial) and the lower (abaxial) surfaces of each leaf starting at the stem and moving towards the tip of the leaf (Figure 2.5).

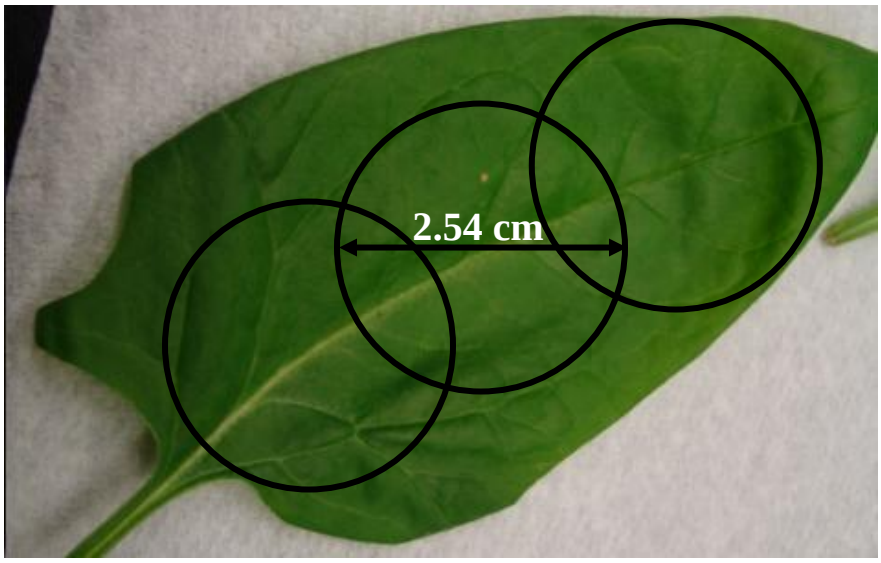

Figure 2.5. Method of measuring color on the front of a spinach leaf. Each circle represents one measurement in which the color parameters of the leaf area contained within the circle are averaged.

A clear film was placed between the sample and the instrument to prevent the sample from damaging the instrument. Although the measurement areas had some overlap (Figure 2.5), as much of the leaf that could be captured was measured. The area captured by each circular measurement area was 6.45 cm^2. The values L*, a*, and b* were collected where L* is the lightness, a* is the red-green scale, and b* is the yellow-blue scale (Figure 2.6).

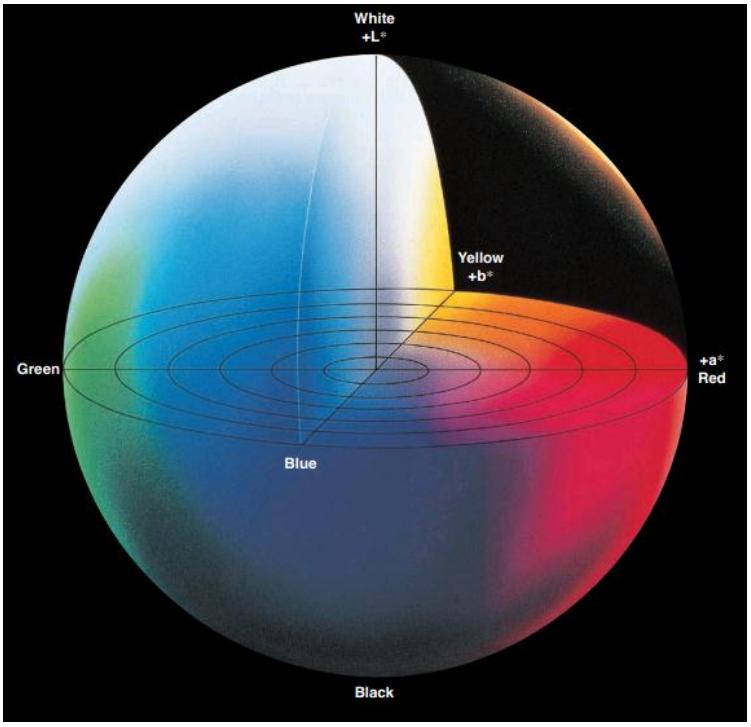

Figure 2.6. L*, a*, and b* values represented on a sphere, taken from (Konica Minolta Sensing, Inc., 2007).

The hue value was determined based on equation 2.2.

$$\text{hue} = \tan^{-1}\frac{b*}{a*} \tag{2.2}$$

For each leaf, the average of six measurements (hue_o) was used to describe the initial color of the leaf. The post treatment value (hue_w) was calculated by taking the worst (lowest hue value) of the three measurements on each side of the leaf. These two values were collected for each of the five leaves and the average of a total of ten measurements for each sample was defined as the hue_w value. The change in hue ($\Delta hue = hue_w - hue_o$) and the hue ratio ($hue_r = hue_w/hue_o$) were the values used to describe the color change of spinach leaves due to the treatment.

2.4.2.2.2. Spinach Qualitative Color Measurement

The visual analysis was performed using photographs of the spinach samples after ClO_2 treatment. The samples were divided into three separate groups, no damage, minimal damage, and substantial damage, based on their physical appearance (figure 2.14).

Damage was defined as any discoloration, whether localized in one area or evenly distributed, present on the abaxial or adaxial surface of the leaves. The stem where the leaf was separated from the plant was not considered in the analysis. Since the ClO_2 interacts with spinach causing a bleaching effect, the discoloration experienced by the samples was a green color changing to a yellow or lighter green color. Samples were placed in the "no damage" group if there was no observable damage on the surface of the leaf. The "minimal damage" label was assigned to leaves if any color discoloration was visible anywhere, and to any extreme, on the surface of the leaves. Samples with one leaf

or more having an estimated area of damage greater than 5% were labeled "substantial damage" regardless of the severity of the damage.

2.4.2.2.3. Color Change during Spinach Storage

Photographs of the leaves were taken and color measurements were performed throughout the storage period to see the effect of ClO_2 treatment on the shelf life of the produce. Leaves that were not inoculated with *E. coli* K-12 but were exposed to ClO_2 were stored in rigid containers, within polyethylene bags, at 4 °C along with a control not exposed to ClO_2. Color measurements were made at day 0, 7, and 14 to asses to color change occurring due to the exposure to ClO_2.

2.4.2.3. Microbial Reduction Studies

2.4.2.3.1. E. coli. K-12 Growth

One day prior to the experiment, 100 ml of TSB growth medium was prepared with chemicals and the media was inoculated with a loop of E. *coli* K-12 from the previously prepared slants stored at 4 °C. The culture was grown on an agitator rotating at 150 rpm inside of an isotemp incubator (11-690-637D; Thermo Fisher Scientific, Pittsburgh, PA, USA) at 37 °C. After approximately 7 h, 10 ml of the culture was transferred into 1100 ml of TSB in a larger flask and allowed to grow for approximately 15 h inside the incubator. Figure 2.7 shows the growth curve of *E. coli* K-12 starting from an initial concentration of $3.4x10^4$ cells/ml. The optical density correlating to each

concentration measured at 600 nm using a spectrophotometer (Spectronic 20 Genesys; Thermo Electron Scientific Instruments Corp., Madison, WI, USA) was also included.

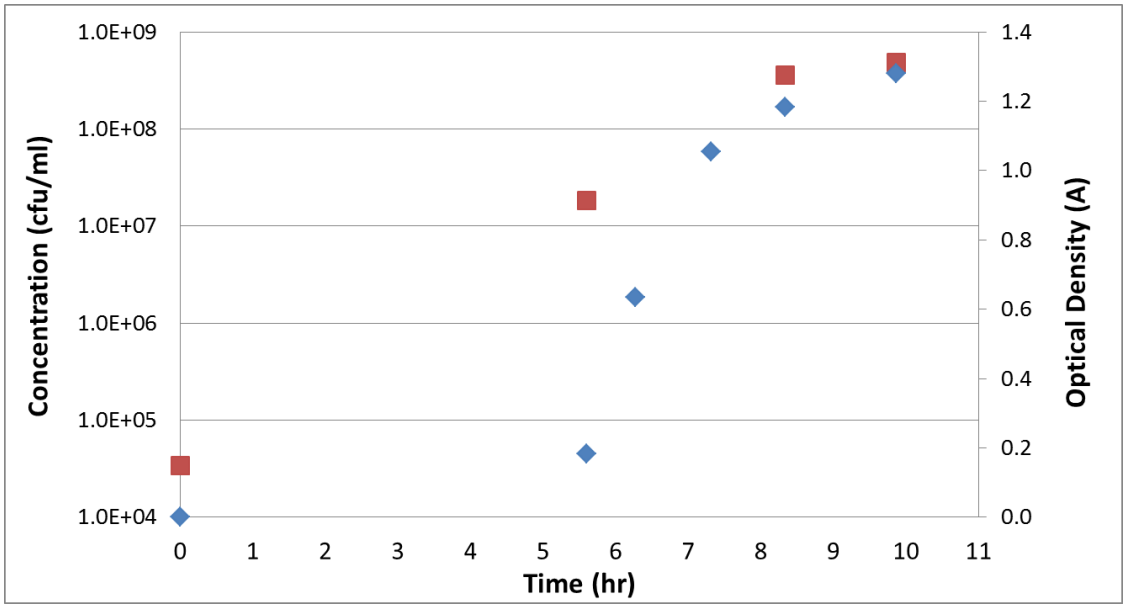

Figure 2.7. Growth curve for E. *coli* K-12 in chemical media from optical density measurement (blue diamonds) and from plate count (red squares).

2.4.2.3.2. Inoculum Preparation

The absorbance of the growing culture was monitored at 600 nm using sterile TSB medium as a reference until a cell growth of at least 10^8 cells/mL was reached. The *E. coli* K-12 was removed from the TSB medium as a pellet by centrifuging (J-21 Series Centrifuge and JA-14 rotor, Beckman Instruments, Inc., Palo Alto, CA, USA) 1,100 mL for 10 minutes at 9,820 xg (8,000 rpm). After centrifugation, the TSB was removed underneath the biosafety hood, leaving the E. coli pellet in the centrifugation bottles. Each centrifuge bottle was filled with 133 ml of peptone water and the pellets were suspended using an agitator or manually shaking the bottle. Dilutions of the sample were

made and the inoculum was plated to determine the initial microbial concentration of the inoculum.

2.4.2.3.3. Surface Attachment of Bacteria

Three separate batches of samples were inoculated; 1) upper leaves submerged in inoculum, 2) lower leaves submerged in inoculum, and 3) color measurement samples submerged in peptone water without bacteria. The upper leaves and lower leaves were submerged separately, though in identical inoculum, to assure each sample had three upper leaves and two lower leaves. A sterile strainer and beaker was chosen for each of the three treatment types. The upper and lower leaves were placed inside separate beakers and agitated manually for 2 minutes. Of the 800 ml of inoculum, approximately three fifths of the bottle was used for the upper leaves, and two fifths for the lower leaves. The samples were quickly removed from the beaker using sterile tongs and shaken to remove excess water. The leaves were then placed on plastic lined paper towels on top of sterile metal trays underneath a biological hood to begin the first 90 minute drying period, during which leaves had time to dry and the bacteria had time to attach to the surface of the produce. After the first drying period, sterile tongs were used to collect the leaves and wash them with water similarly to the inoculation procedure. This washing step simulates a wash that would occur in industry to remove organic matter from the surface of the produce. The leaves were dried for 90 minutes for a second time. After the second drying period, the two samples used for color analysis were removed from underneath the hood and photographed. The mass of five leaves was recorded for each of

the remaining four samples being exposed to ClO_2. The six trays (4 inoculated and 2 not inoculated) were then randomly positioned on an autoclaveable test tube rack (Figure 2.8) and placed inside the sample chamber (Figure 2.9).

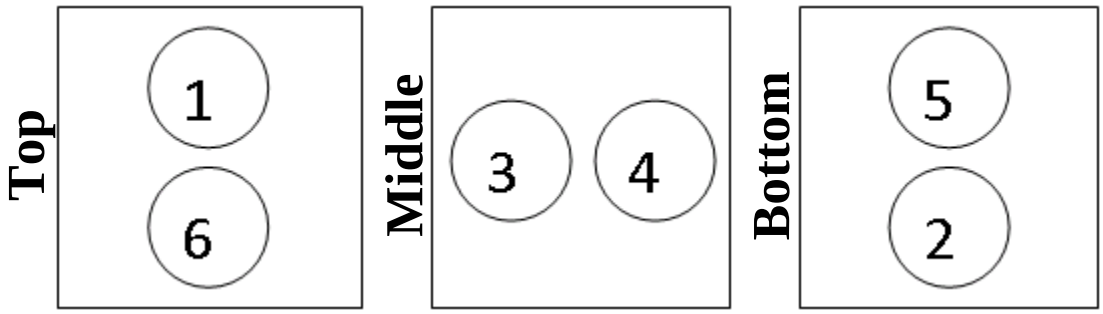

Figure 2.8. Example of sample positioning on top, middle, and bottom tiers of the rack from left to right.


Figure 2.9. Six samples randomly positioned on an autoclaveable test tube rack (left); samples inside of the sample chamber which is located inside the treatment chamber (right).

The empty sample chamber was tested for initial conditions (temperature, relative humidity, open voltage, and LED current) prior to loading the spinach samples.

2.4.2.3.4. ClO_2 Treatment

The sample chamber was sealed and the fans were turned on to ensure ClO_2 uniformity inside the treatment chamber. The 35% $NaClO_2$ and 6N HCl were then added into the beaker to generate desired ClO_2 concentration (see section 1.3.2.2.) and the treatment chamber was sealed. Once the concentration within the treatment chamber reached a steady state value, the sample chamber was opened exposing the samples to the ClO_2 gas for the assigned treatment time. Once the treatment was finished, the treatment chamber was opened underneath the laminar hood, purging the ClO_2 gas from the treatment chamber into the chemical hood.

2.4.2.3.5. Sample Plating

The samples were removed from the sample chamber and placed underneath the sterile biosafety hood to prevent outside contamination. Seven ounce polyethylene bags (Whirl-Pak, B00992; Nasco, Atlanta, GA, USA) were prepared with the proper amount of neutralized peptone water to create a ten-fold dilution. The samples were aseptically transferred into a Whirl-Pak and the bag was placed in a small cooler with icepacks evenly distributed throughout. The samples were stomached, two bags at a time, for 2 minutes to homogenize (this will serve as the 10^{-1} dilution). The samples were then serially diluted in 2 ml microcentrifuge tubes (02-681-258; Thermo Fisher Scientific, Pittsburgh, PA) by placing 0.1 mL of the previous dilution into 0.9 mL of peptone water until the desired dilutions were obtained (typical dilution scheme seen in Table 2.1). The

pipette tip was changed and the previous dilution mechanically agitated (Maxi Mix II; Thermolyne Corp., Dubuque, IA, USA) before each new aliquot was made to produce accurate dilutions. Once the dilutions were complete, the centrifuge tube being plated was mechanically agitated and 100 µL of the chosen dilution was dispensed onto agar (BP1423; Thermo Fisher Scientific, Pittsburgh, PA, USA) containing TSB medium. Glass plating beads (#C400100; Genlantis Inc., San Diego, CA, USA) (approximately 12-15) were used to evenly distribute the dilution onto the plate. When the 10^0 dilution was being plated, a plate with 0.4 ml and two with 0.3 ml of sample were made. The plates were incubated at 37 °C for 18-24 h.

2.4.2.3.6. Enumeration

After incubation for 18-24 h, the plates were removed from the incubator and enumerated using standard enumeration techniques found in the FDA Bacteriological Analytical Manual (Maturin and Peeler, 2001). Duplicate, and in some cases triplicate, plates were made for every dilution that was plated. Plates were counted that contained between 25 and 250 CFU/plate. In the case of the 10^0 dilution, the cell counts on all three plates were added together. Plates within the same dilution were averaged. If both plates from two separate dilutions fell within the countable range, then the average of those two dilutions were taken (Sutton, 2011). The microbial concentration of the dilution was then back calculated from the plate count.

2.4.2.4. Experimental Design and Data Analysis

Concentration-exposure time combinations were chosen based on the literature cited in tables 2.3 and 2.4. Since kinetic studies require the ClO_2 concentration to be held constant, four concentrations were chosen (2.05, 3.75, 4.73, and 5.86 mg/l) and different exposure times were tested. In addition to the points used in the kinetic study, several other combinations were chosen to test the physical and microbial degradation in areas not covered by the kinetic study. The experimental design included 31 resulting test points. Color measurements were performed for all of the points in the resulting design space (Figure 2.10).

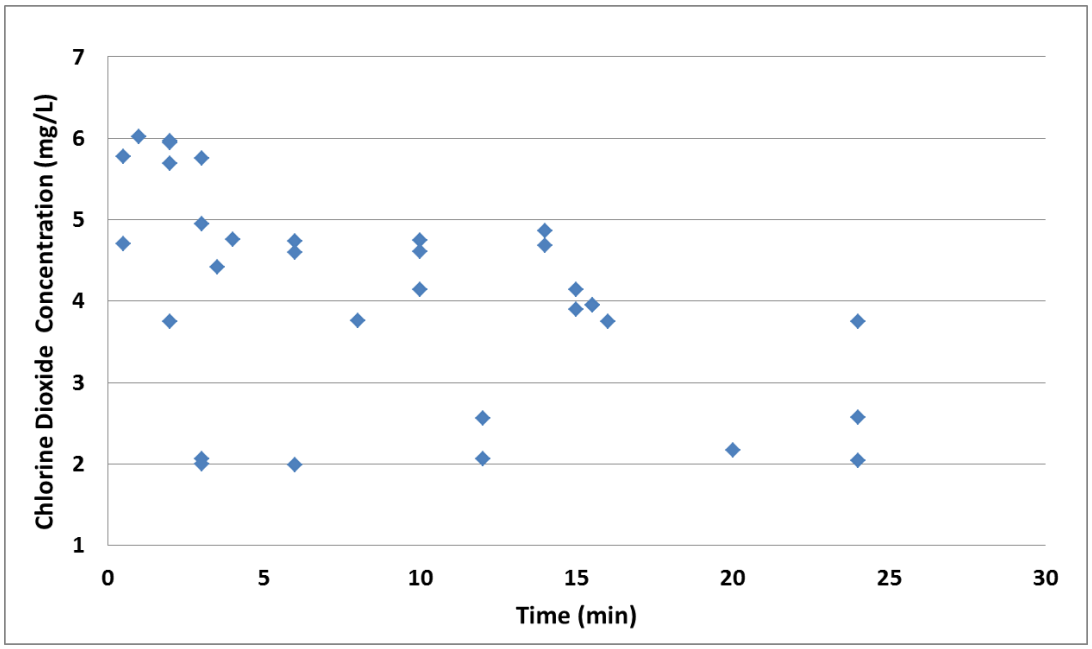

Figure 2.10. Data points for color measurements for all of the concentration-exposure time combinations for ClO_2 treatment.

The hue change and hue ratio at each point was the mean of 5 or 10 leaves measured front and back then averaged together. Several points were then replicated 1-2 times and their differences were tested using analysis of variance with significances at $p<0.05$. Hue changes were used to quantify color change throughout the majority of the study because the average initial hue angles were similar. The hue ratio was used when the differences between the initial hue angles were greater than 4° and when performing kinetic analysis which utilized the natural logarithm of the hue ratio.

Table 2.6. Four ClO_2 concentrations used for kinetics studies of color change of spinach leaves.

ClO_2 Concentration (mg/l)	**Time (min)**
2.05 ± 0.06	3
	6
	12
	20
	24
3.75 ± 0.01	2
	8
	15.3
	24
4.73 ± 0.11	0.5
	3.5
	6
	10
	14
5.86 ± 0.13	0.5
	1
	2
	3

2.5. Results and Discussion

2.5.1. Physical Effects of Chlorine Dioxide Exposure

2.5.1.1. Variability of Spinach Leaves

Although the spinach plants were grown under controlled conditions, differences between the leaves still occurred within each plant. Upper leaves appeared darker and had an elongated elliptical shape, while the lower leaves appeared lighter and rounder. This may be a developmental property of spinach leaves depending on the amount of sunlight a specific leaf receives during growth. Figure 2.11 shows the difference between upper and lower leaves.

Figure 2.11. Upper leaves (left) and lower leaves (right) of spinach plant.

The average L*, a*, b*, and H_0 and standard deviations are shown for both upper and lower leaves in table 2.7.

Table 2.7. Initial color values for upper and lower leaves.

Leaf and Side		L*	a*	b*	H_0
Upper Adaxial	*average*	49.41 a	-5.09 a	7.53 a	125 a
	SD	1.64	0.70	2.12	3.86
Upper Abaxial	*average*	54.14 b	-5.96 b	12.58 b	115.49 b
	SD	1.54	0.42	1.44	1.39
Lower Adaxial	*average*	51.71 c	-6.08 bc	11.38 b	119.54 c
	SD	2.42	1.10	4.14	4.82
Lower Abaxial	*average*	56.71 d	-6.42 c	14.99 c	113.37 d
	SD	2.16	0.53	2.18	1.41

Values in each column not connected by the same letter are significantly different at $p<0.05$.

Significant statistical differences were observed between all four measured values between the top and bottom of the upper and lower leaves. The difference in initial color is confirmed by the L* value (lower leaves are lighter), a* (lower leaves are greener), b* (lower leaves are more yellow), and H_0 (lower leaves are greener).

2.5.1.2. Color Degradation due to ClO_2 Treatment

Figure 2.12 shows the average concentration at each exposure time used for the kinetics study.

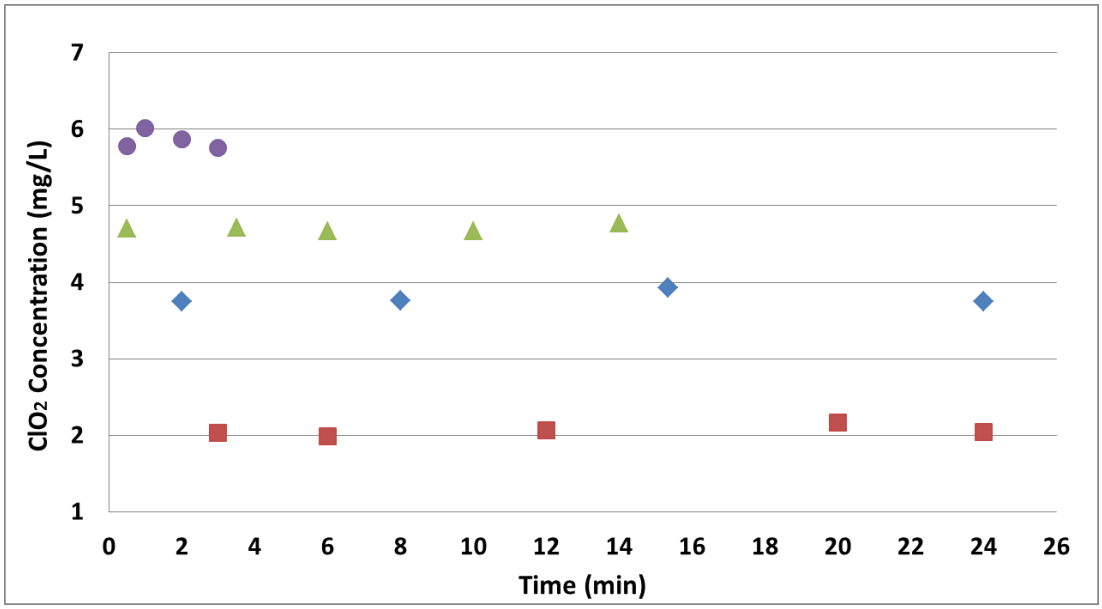

Figure 2.12. Average ClO_2 concentrations for trials used in the kinetics study; 2.05 mg/l (red squares), 3.75 mg/l (blue diamonds), 4.73 mg/l (green triangles), 5.86 mg/l (purple circles).

Chlorine dioxide exposure caused discoloration on the surface of the leaves, changing portions of the leaf from green to yellow. The worst hue change from the sample (ΔH_w) for each trial are shown in table 3.8. The results show that the change in hue increases with increasing exposure time at any ClO_2 concentration and with increasing ClO_2 concentration for any given treatment time, excluding 2.05±0.06 mg/l ClO_2 at 12 and 24 minutes. The relationship between the two variables can be seen by comparing the changes in hue among various combinations. Mahmoud et al. (2008) reported a ΔH = -22.57 (H_0 = 106.37, H_f = 83.80) of 5cm^2 iceberg lettuce leaves exposed to 5.0 mg/l at 90-95% relative humidity for 2 minutes. Vandekinderen et al., (2009) reported a ΔH = -8.55 (H_0 = 104.28, H_f = 95.73) of 2 kg iceberg lettuce exposed to 1.54 mg/l at 90.5±1% relative humidity for 9.5 minutes. These studies showed much larger changes in hue than

the results presented in this study. While there was a difference in initial hue and properties between spinach and iceberg lettuce, a major difference between the two studies was the relative humidity. Due to its high solubility, ClO_2 gas may dissolve in water on the surface of the leaves when high humidity environments allow for condensation. If ClO_2 dissolves on the surface of the samples, the prolonged contact with ClO_2 may cause additional damage not seen in an environment ranging from 22-53% relative humidity. The kinetic analysis of the data in Table 2.8 is presented in section 2.5.2.

Table 2.8. ΔH_w for each of the points shown in Figure 2.14.

Concentration mg/L						
5.86±0.13	Time (min)	0.5	1	2	3	
	ΔH_w	-0.74127	-1.11451	-3.04887	-3.15747	
4.73±0.11	Time (min)	0.5	3.5	6	10	14
	ΔH_w	-0.70757	-1.03879	-1.87732	-2.06307	-3.31288
3.75±0.01	Time (min)	2	8	15.3	24	
	ΔH_w	-0.08613	-0.95193	-2.30817	-4.81822	
2.05±0.06	Time (min)	3	6	12	20	24
	ΔH_w	-0.24626	-0.27304	0.074649	-1.28329	-0.41134

2.5.1.3. The ClO_2 Concentration-Exposure Time Design Space

The color data of spinach were analyzed both visually and quantitatively. Figure 2.13 gives an example of the visual ratings given to each set of leaves.

Figure 2.13. Photographs of spinach leaves assigned “no damage” in 1 (1.99 mg/l, 6min) and 2 (4.42 mg/l, 3.5 min); “minimal damage” in 3 (2.16, 20 min) and 4 (4.60, 10 min); and “substantial damage” in 5 (4.87, 14 min) and 6 (3.75, 24 min).

Figure 2.14 was constructed classifying the spinach leaves as no damage, minimal damage, or substantial damage.

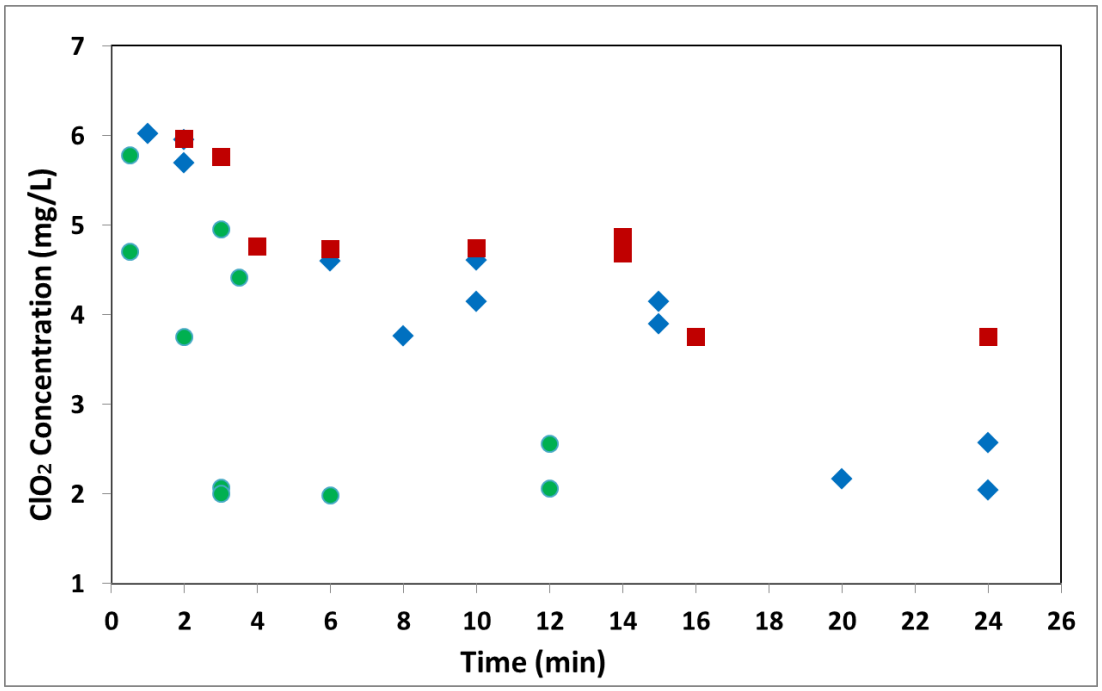

Figure 2.14. Visual ratings of post-treatment leaves, no damage (green circles), minimal damage (blue diamonds), and substantial damage (red squares).

The three regions of classifications were also in agreement with the quantitative color measurements. Samples that experienced a hue value change of Δhue greater than -1.5 were considered to have "substantial damage". It was found that out of the 31 samples shown in Figure 2.15, only two did not meet this criterion (-1.49 was judged substantial damage, and -1.53 was judged minimal damage, shown in Appendix I). Figure 2.15 shows the three regions defined based on the color measurements of the spinach leaves after ClO_2 treatment.

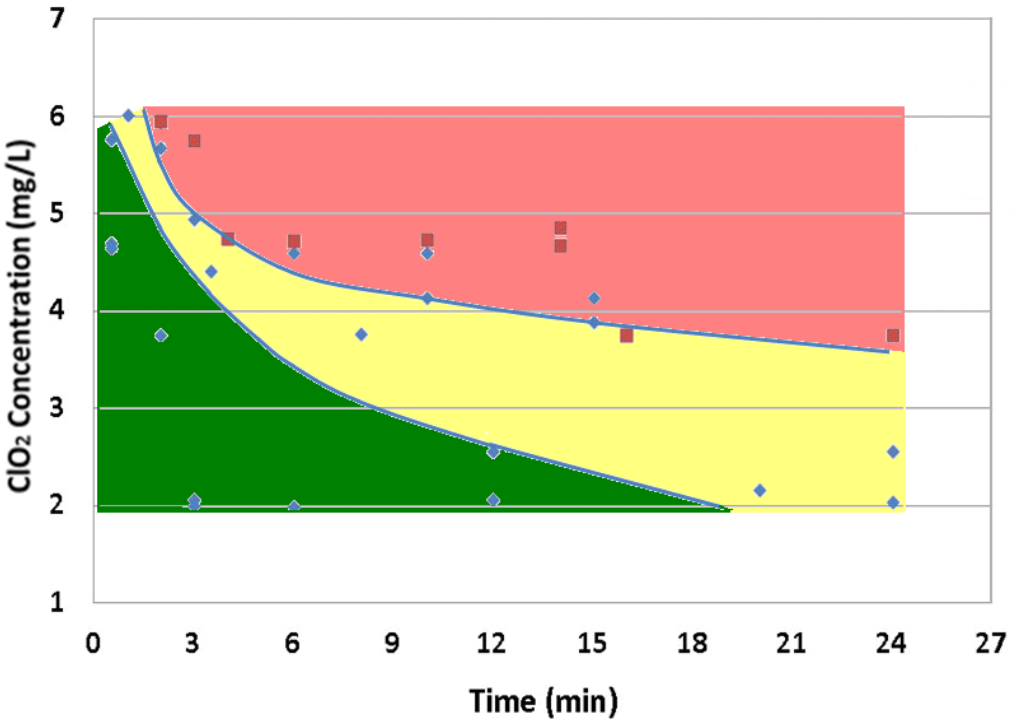

Figure 2.15. Three distinct regions, no damage (green), minimal damage (yellow), substantial damage (red).

Such defined regions can be utilized to predict the acceptability of spinach leaves after a particular concentration-exposure time combination. Furthermore, the state diagram developed based on the color measurements was utilized as a screening tool when selecting treatments for microbial studies.

2.5.2. Color Degradation Kinetics for Spinach Treated with ClO_2

In order to determine the rate at which physical degradation occurs as a function of time on spinach treated with ClO_2 gas, experiments were designed to collect color degradation as a function of time. The before and after images of the spinach leaves used in the kinetic study are shown in figure 2.16.

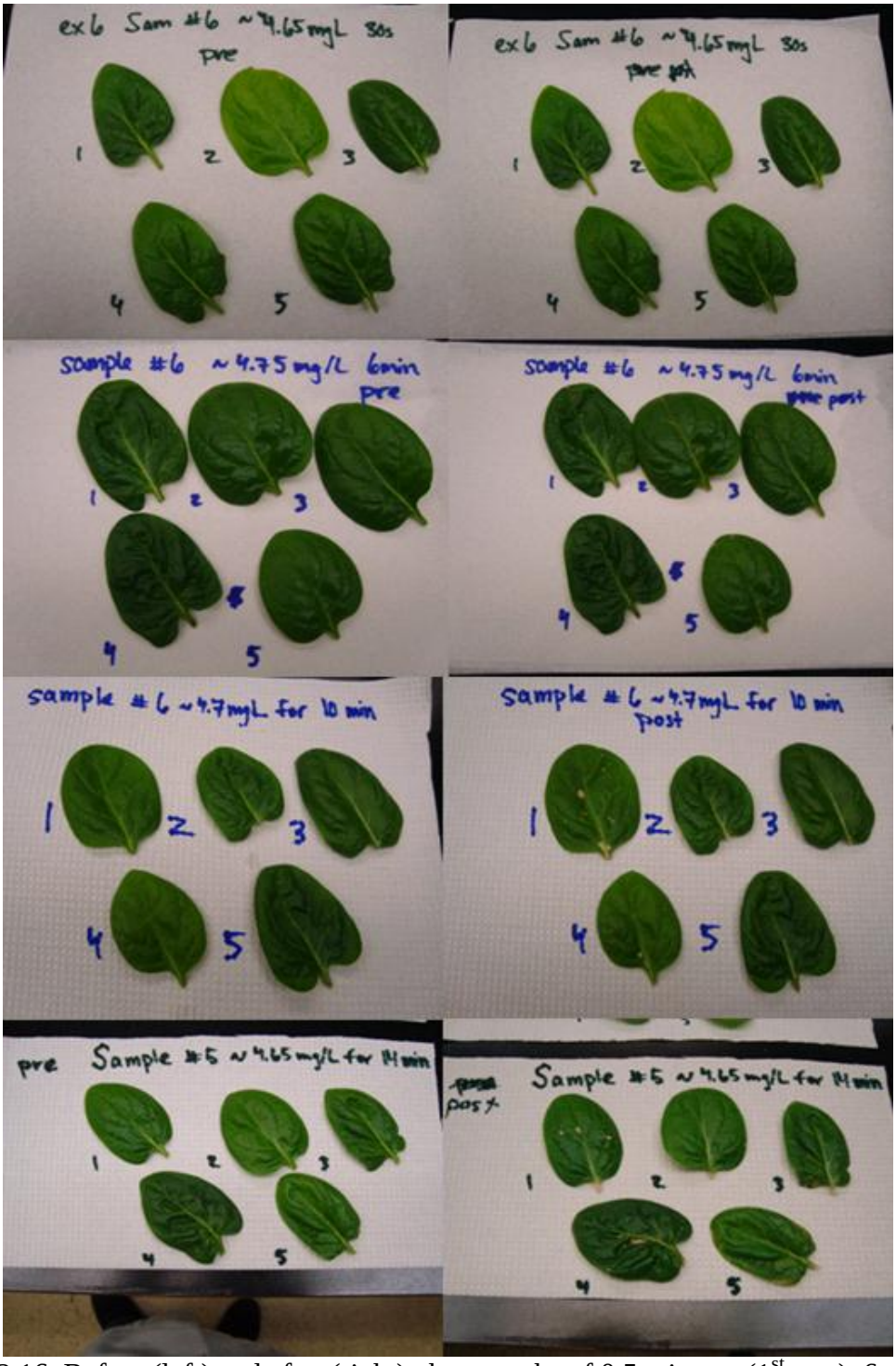

Figure 2.16. Before (left) and after (right) photographs of 0.5 minutes (1st row), 6 minutes (2nd row), 10 minutes (3rd row), 14 minutes (4th row) exposures at approximately 4.73 mg/l.

The equation was fitted to the color data to calculate the rate constant of degradation (equation 2.3).

$$\ln \frac{H}{H_0} = -kt \qquad (2.3)$$

The rate of constant color degradation (k) was determined from the slope of $\ln(H_w/H_o)$ as a function of time (Figure 2.17).

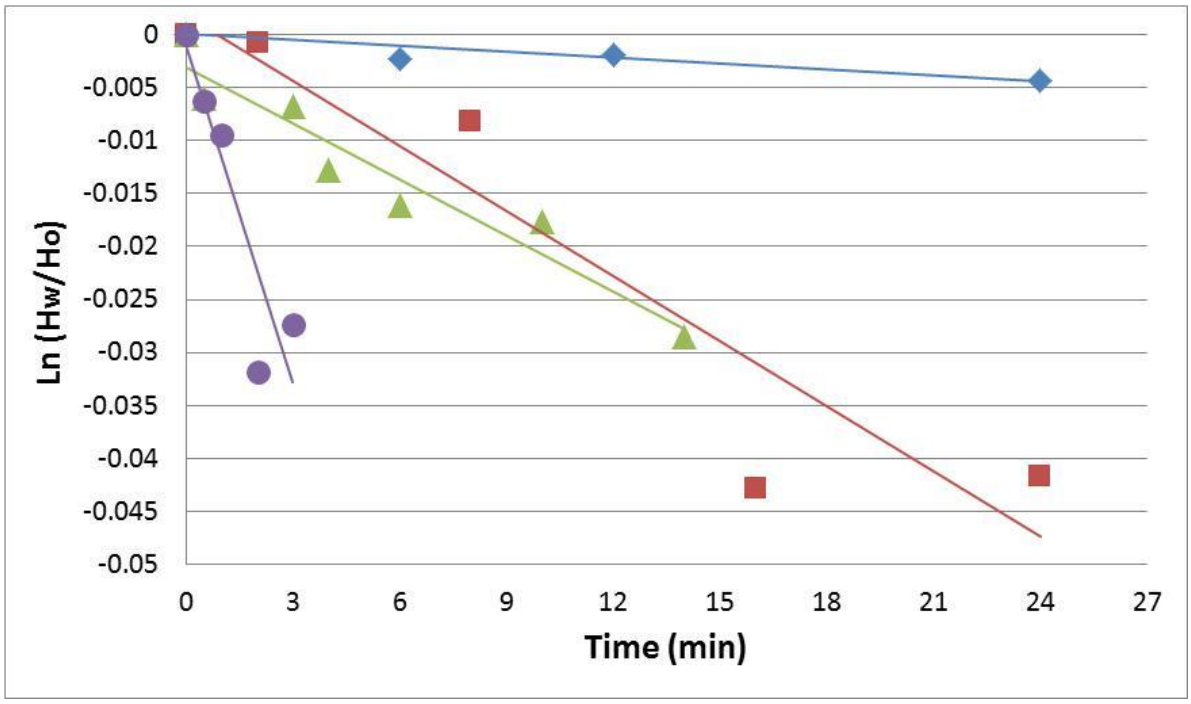

Figure 2.17. Kinetic plot of four concentrations for which the slope is equal to the rate constant of color degradation. Concentration of 2.05 mg/l (blue diamonds), 3.75 mg/l (red squares), 4.73 mg/l (green triangles), and 5.86 mg/l (purple circles).

The rate constants of color degradation were calculated at four ClO_2 concentrations as shown in figure 2.12 and listed in table 2.6. As ClO_2 concentration increases, the rate constant of color degradation increases indicating a shorter exposure time will be

necessary to cause color change. From equation 2.4 the amount of time it would take to observe a ten-fold decrease in hue value (D-value).

$$D\text{-}value = \frac{2.303}{k} \tag{2.4}$$

According to table 2.9 it would take more than 3 days of 2 mg/l ClO_2 exposure to cause a ten-fold decrease in the hue value. Recall that a change in hue of greater than -1.5° signifies that the sample was substantially damaged and the average initial hue was 122.8°, so a small reduction in the hue ratio signifies major color change. In fact, since hue angle is not a linear scale, spinach leaves will not likely approach zero as time approaches infinity. For this reason, the D-value is not a useful parameter when discussing the kinetics of color change and should instead only be used when discussing microbial degradation on spinach leaves. Table 2.9 shows the rate constants of the hue degradation at each of the four concentrations tested.

Table 2.9. The four rate constants determined from the kinetics studies of hue value change as a result of ClO_2 treatment.

Concentration	**Rate constant (k)**	**D-value**
mg/l	min^{-1}	min
2.05 ± 0.06	0.0002	11515
3.75 ± 0.01	0.002	1152
4.73 ± 0.11	0.0018	1279
5.86 ± 0.13	0.0105	219

Plotting the four rate constants describing the hue change for treated spinach against concentration the activation energy was determined from the slope shown in Figure 2.18.

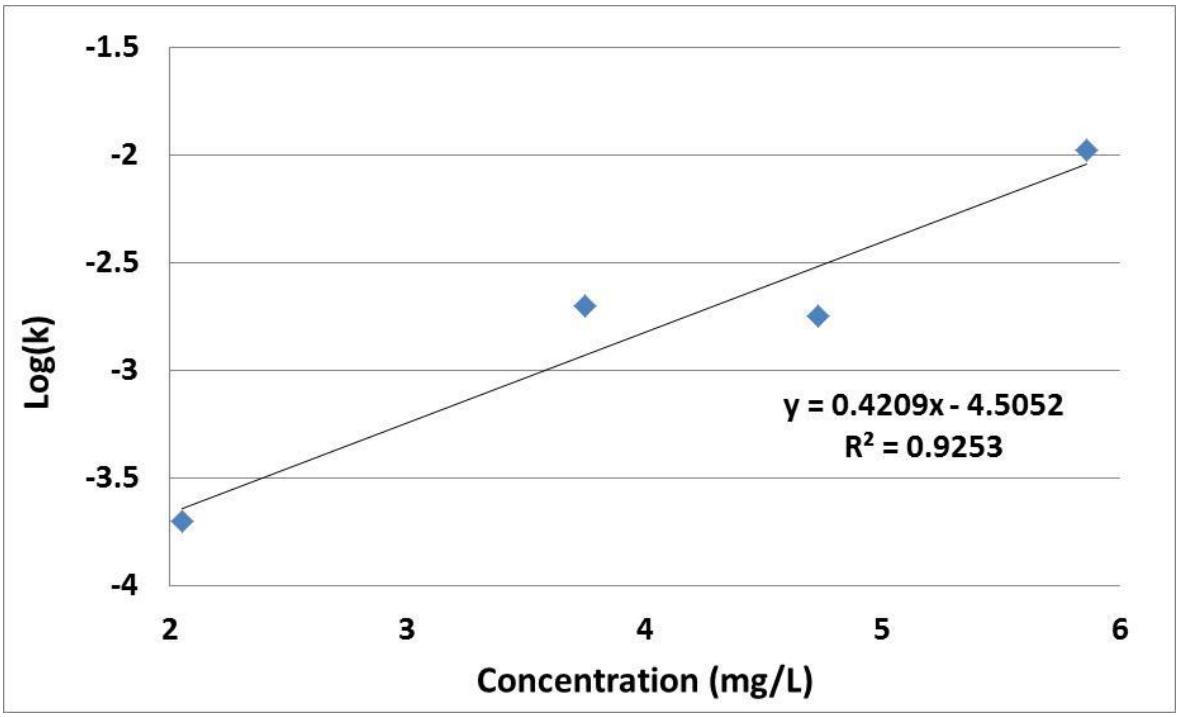

Figure 2.18. The rate constant for the change of hue angle as a function of ClO_2 concentration.

The relationship described in Figure 2.18 can be used to predict the rate constant of hue change at any concentration over the 2-6 mg/l range that could be applicable for fresh produce sanitization. The flexibility of equation 2.5 allows the hue change, concentration, or exposure time to be determined if two of the variables are known.

$$\log(k) = -0.4209c + 4.5052 \qquad (2.5)$$

Therefore, if a maximum allowable hue change can be defined, all of the possible concentration-exposure time combinations can be determined.

2.5.3. Microbial Effects of Chlorine Dioxide Exposure

Seven concentration-exposure time combinations were chosen to investigate the microbial reduction attainable (Table 2.10), and to develop a model for the kinetics of microbial reduction at 4.73 mg/l ClO_2 concentration (figure 2.20).

Table 2.10. Microbial reduction and change in hue values caused by various concentration and exposure time combinations.

Trial #	**Concentration** mg/l	**Exposure Time** min	**Inocula** CFU/ml	**Inoculated, Not Treated** CFU/g	**Inoculated, Treated** CFU/g	**Log Reduction** log CFU/g	**Change in Hue Values** ΔH
1	2.05±0.06	3	7.05 ± 0.23	4.73 ± 0.29	3.53 ± 0.12	**1.20**	ΔH_a = -0.79
2	2.05±0.06	20	7.91 ± 0.23	5.52 ± 0.53	3.24 ± 0.18	**2.27**	ΔH_a = -0.62 ΔH_W = -1.28
3	4.73±0.11	0.5	7.50 ± 0.06	5.36 ± 0.39	4.08 ± 0.27	**1.28**	
4	4.73±0.11	0.5	8.28 ± 0.39	5.28 ± 0.52	4.05 ± 0.39	**1.18**	ΔH_a = -0.33 ΔH_W = -0.71
5	4.73±0.11	6	8.71 ± 0.03	5.41 ± 0.50	3.37 ± 0.17	**2.04**	ΔH_a = -0.03 ΔH_W = -0.64
6	4.73±0.11	10	8.21 ± 0.03	5.80 ± 0.45	3.57 ± 0.12	**2.23**	ΔH_a = -0.96 ΔH_W = -1.53
7	4.73±0.11	14	7.45 ± 0.02	4.92 ± 0.46	2.48 ± 0.06	**2.37**	ΔH_a = -1.95 ΔH_W = -3.40
8	5.86±0.13	2	8.38 ± 0.00	5.46 ± 0.40	4.67 ± 0.32	**0.79**	ΔH_a = -0.70 ΔH_W = -1.37

Treatment number does not correspond to the order the trials were conducted in.

The average change in hue (ΔH_a, all six of the post-treatment measurements minus all six of the pre-treatment measurements for each leaf) and ΔH_w are given in Table 2.10. Figure 2.19 shows the log reduction and color change data on the state diagram that was developed based on the color change data.

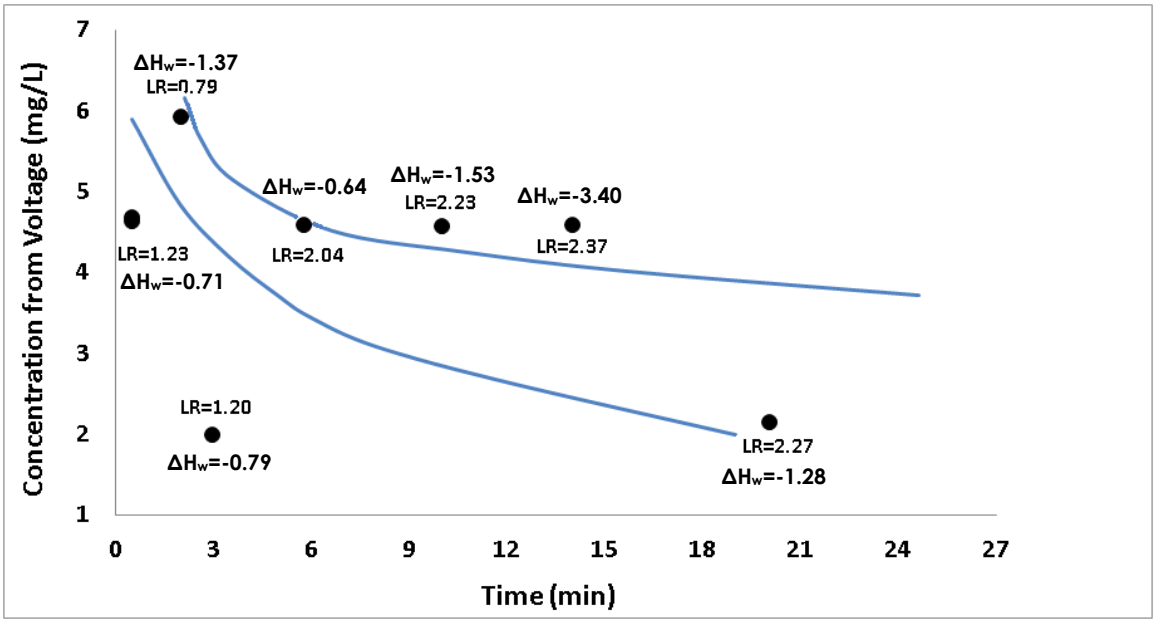

Figure 2.19. Log reductions in microbial population and corresponding hue angle changes for the points used in microbial study.

The log reductions reported in table 2.4 were higher than the *E coli.* O157:H7 reductions reported under similar treatment conditions by Sy et al. (2005) and Mahmoud et al. (2007) for iceberg lettuce, and Neal et al. (2012) for spinach. Sy et al. (2005) reported a 1.57 log CFU/g reduction with 20.5 min exposure at 4.1 mg/l ClO_2 gas concentration, while this study observed a 2.37 log CFU/g reduction with 14 min exposure at 4.68 mg/l ClO_2 concentration. Mahmoud et al. (2007) reported a 3.44 log

CFU/g reduction in iceberg lettuce with 10 min exposure at 5 mg/l ClO_2, while this study observed a 2.23 log CFU/g reduction with 10 min exposure at 4.60 mg/l ClO_2 concentration. Neal et al. (2012) reported a 0.7 log CFU/g reduction in spinach with 60 min exposure at 2.1 mg/l ClO_2, while this study observed a 2.27 log CFU/g reduction with 20 min exposure at 2.16 mg/l ClO_2 concentration. Differences in bacterial reductions may be, in part, due to the strain of *E. coli* used and the inoculation method. Although spot inoculation may simulate point contamination from animal or human contact, submerging the sample in the inoculum more closely represent cross contamination during fresh produce processing (Beuchat et al., 2001). Since Johnston et al., (2005) has shown an increase in microbial concentration during fresh produce processing, inoculation by submersion may be a better method of inoculation when analyzing the effectiveness of sanitizers. Submersion becomes especially important for leafy green vegetables with more pores and crevices. Submersion allows bacteria to penetrate into damaged areas, including the stem, and gives bacteria access to stomata. Table 2.4 shows a higher bacterial reduction in those studies on leafy green vegetables that used spot inoculation as opposed to submerging the samples in the inoculum.

The data also indicate that bacterial reduction rate changes during the exposure time (Figure 2.20). Two regions with different rate of bacterial reduction are apparent up to 0.5 min and between 0.5 and 14 minutes. This is consistent with many studies including Singh et al. (2002) who reported a log reduction of 0.73 CFU/g after 5 min, 1.17 CFU/g after 10 min (62.4% increase compared to 5 min interval), and 1.25 CFU/g after 15 min (9.4% increase compared to 10 min interval) all at a ClO_2 concentration of

0.5 mg/l. Since most of the bacterial inactivation occurs near the beginning of the ClO_2 exposure, there may be advantages to using shorter exposure times when sanitizing fresh produce. Reduced exposure time may help to preserve important quality parameters of leafy greens throughout the ClO_2 treatment.

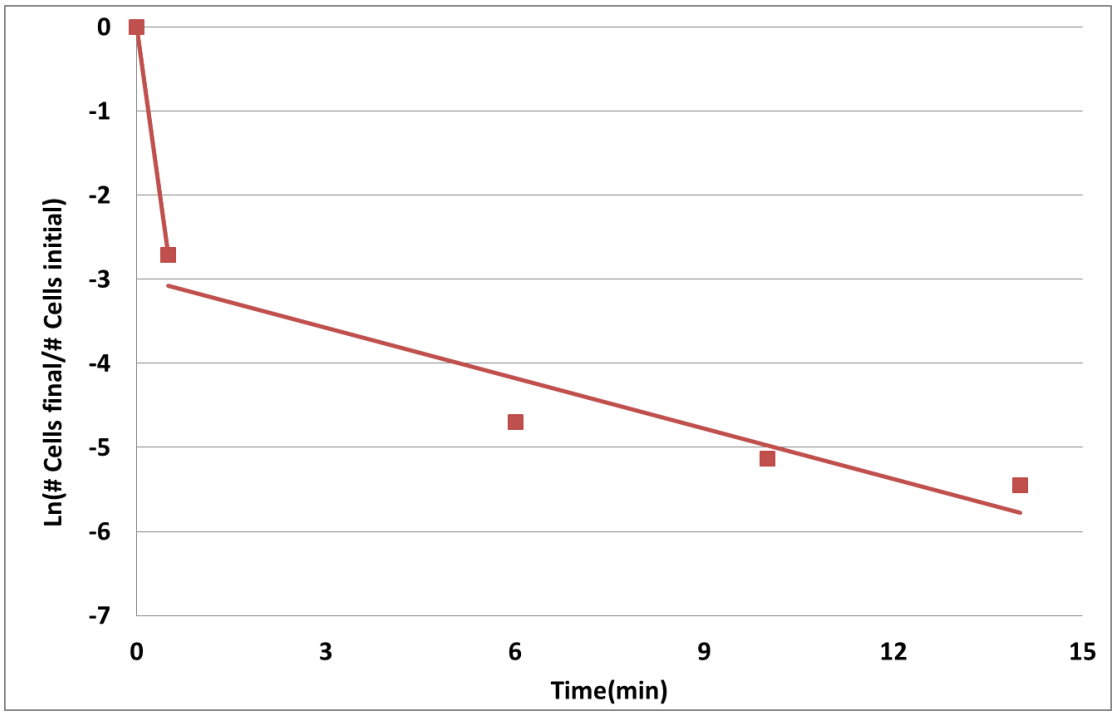

Figure 2.20. Different rates of microbial reduction in two different ranges of exposure time to 4.73±0.11 mg/l.

Figure 2.21 shows both the log microbial reduction and the log hue change at 4.73 mg/l.

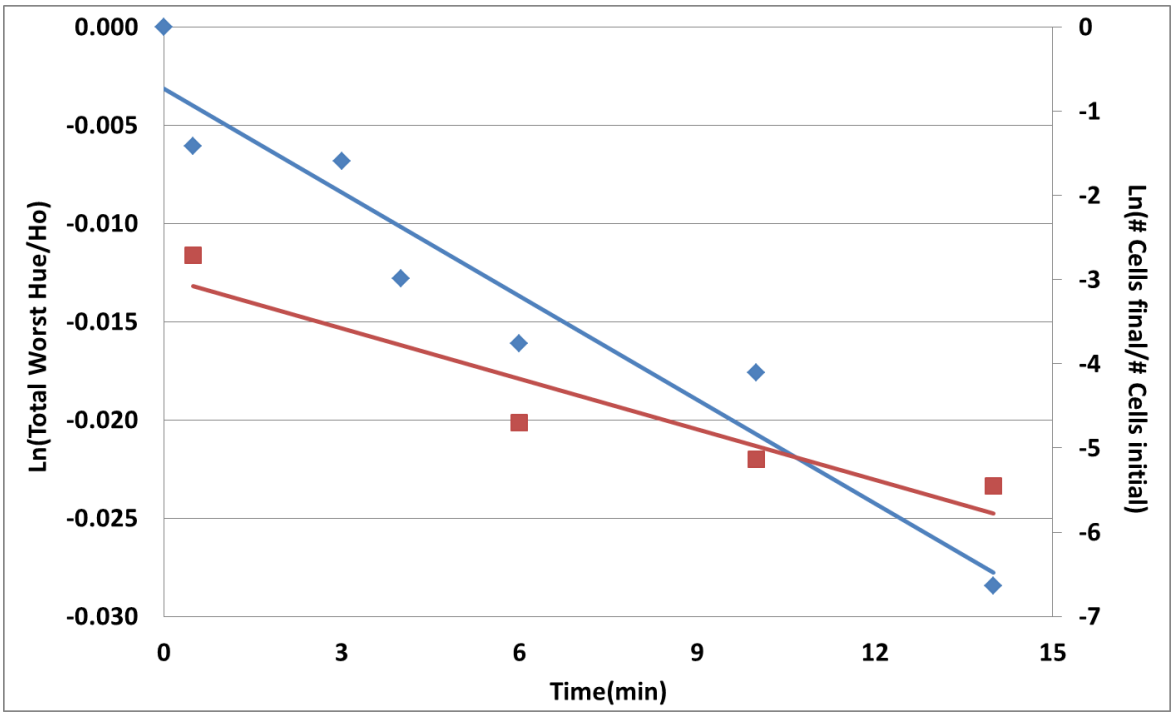

Figure 2.21. Treatments used to determine the hue ratio (blue diamonds) and microbial ratio (red squares) with respect to time of exposure to 4.73±0.11 mg/l chlorine dioxide gas.

Figure 2.21 can be used to determine the treatment time at a given ClO_2 concentration for a required bacterial reduction and then to predict whether the color change after the treatment would be acceptable. The equation of the microbial data points are shown in equation 2.6 as derived from Figure 2.21.

$$\ln\frac{N}{N_0} = -0.2003t - 2.973 \qquad (2.6)$$

where N is the number of cells after ClO_2 treatment and N_0 is the number of cells before ClO_2 treatment. From the rate constant of k = 0.2003 min^{-1} a D-value of 11.50 min was found. This means that at 4.73 mg/l, an increase in exposure time of 11.50 minutes is required to attain one additional log reduction of the microbial concentration. Mahmoud et al. (2008) reported a D-value of 2.9 min at a concentration of 5.0 mg/l on lettuce

inoculated with *E. coli* O157:H7 suggesting microorganisms were more easily destroyed in that study. While higher D-values were seen in other studies, treatment 4 (Table 2.4) can be used to achieve a 2.04 log CFU/g reduction of *E. coli* K-12 while not impacting visual appearance and having a hue angle change of only -0.64. Chlorine dioxide treatments that yield results similar to this have the potential to be much better than the current sanitation practices.

2.5.4. Shelf Life Study Over 14 Days

For four studies exposed to concentrations of approximately 4.73 mg/l the samples were stored after the post-treatment and the color was measured during the storage after 7 and 14 days. The four hue angles (before treatment, immediately after treatment, 7 days, and 14 days) for each sample and their control are shown in Figure 2.22. The photographs of select leaves over the two week period are shown in Figures 2.23, 2.24, and Appendix M. Control samples were harvested and stored with the treated samples, but were not inoculated with *E. coli* K-12 nor treated with ClO_2 gas.

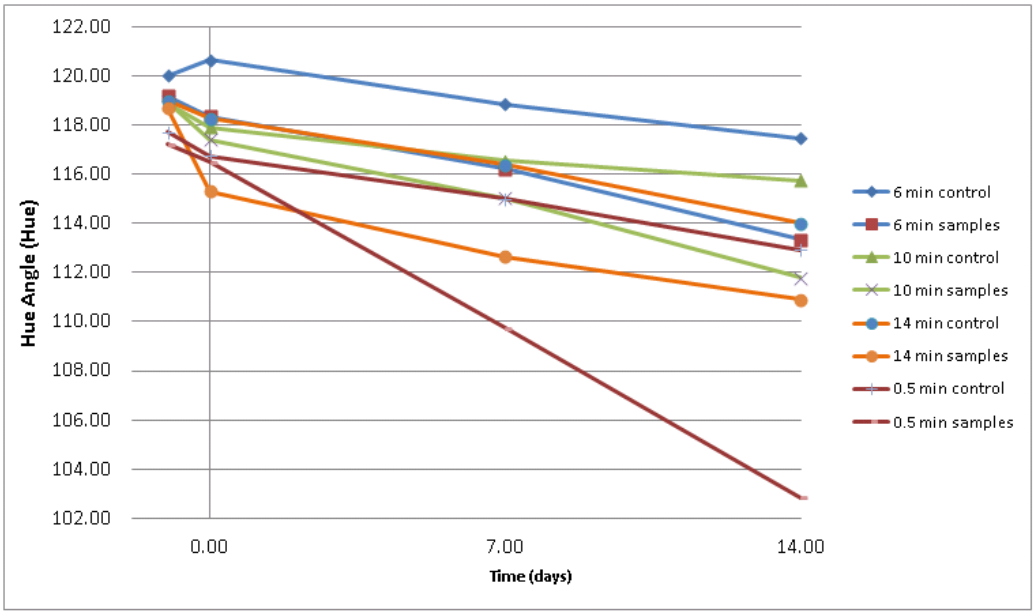

Figure 2.22. Hue angle of leaves as a function of time during storage. ClO_2 treatment was applied at a concentration of 4.73 mg/l for varying times of 0.5, 6, 10, and 14 min. (Data contained in Appendix K).

From the data it can be seen that in all cases the greater the length of exposure the larger the drop in hue angle after the treatment. The hue angle reduction immediately after the treatment, was reflective of the extent of damage on the leaves due to the treatment. The decrease in hue angle appears to be linear over the 14 day period. Regardless of the initial damage to the color of the leaves due to varying exposure times, the rate of color change during the storage (slope of the lines) were similar for all the samples treated at the same ClO_2 concentration of 4.73 mg/l. Because the control and treated samples behave similarly during the duration of the 14 day period it can be speculated that there is no effect of residual ClO_2 on the hue angle during storage.

It is important to note that since the spinach had been grown in a greenhouse, under controlled conditions, the natural microflora present on the spinach was unlikely to

cause extensive spoilage. Because the initial microbial population and diversity were likely lower in the greenhouse than field grown produce, the controls may have experienced reduced degradation. If the control and treated spinach had been inoculated with additional bacteria or fungi, then the treated spinach may have showed less color loss than the untreated spinach.

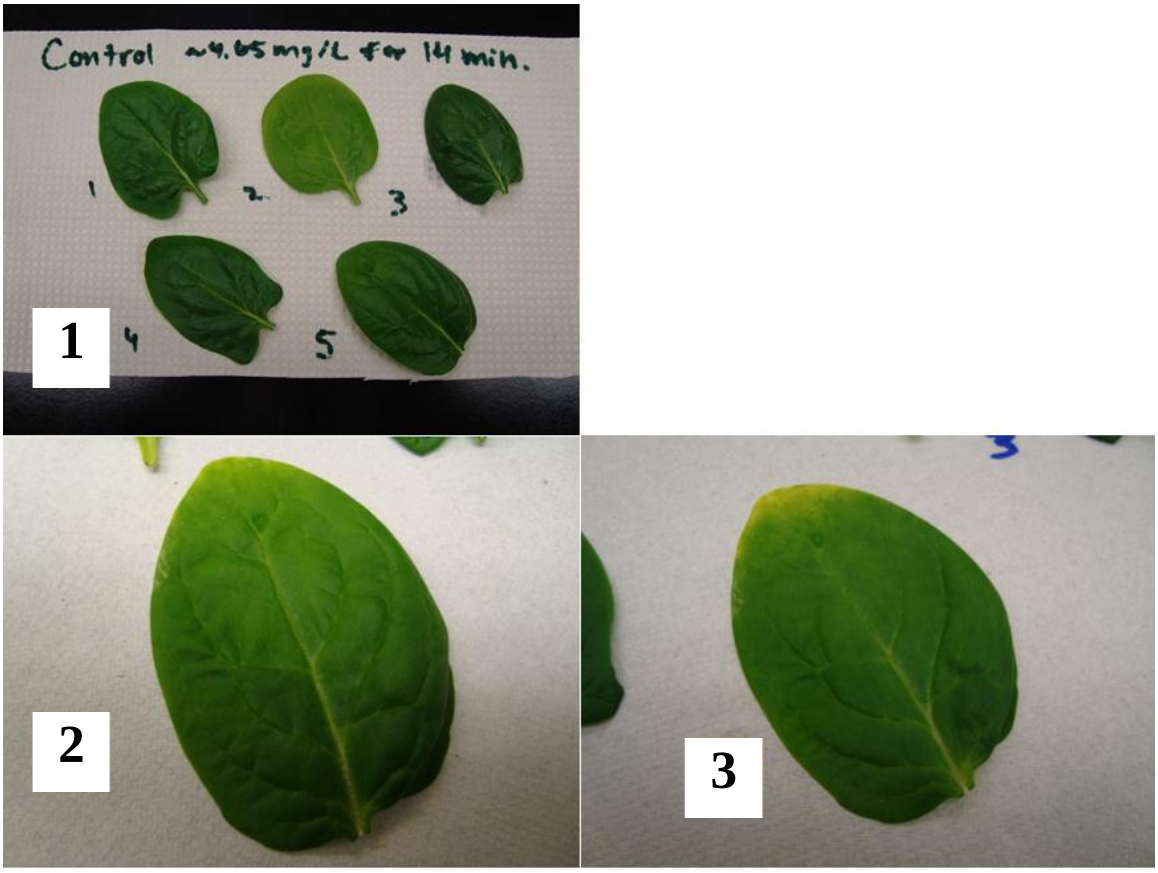

Figure 2.23. Photographs of untreated control in the 14 minute exposure and 4.68 mg/l ClO_2 treatment at 0 days (1), 7 days (2), and 14 days (3).

Figure 2.24. Photographs of leaves treated with 4.68 mg/l ClO_2 for 14 minutes at 0 days (1), immediately after exposure (2), 7 days (3), and 14 days (4).

2.6. Conclusions

Current fresh produce processing methods are inadequate to assure the safety of fresh food consumers. Although the FDA does not specify requirements regarding the level of microbial reduction that has to be achieved by processors, a <1.7 log reduction (1.5 log reduction on tomatoes (Bhagat et al., 2010) 1.7 and 1.2 log reduction on lettuce and cabbage, respectively (Han et al., 2001)) was not considered to be sufficient to

prevent future outbreaks. Gaseous sanitizers appear to be the only feasible method of inactivating pathogenic bacteria regardless of their location on the surface or within the produce.

Chlorine dioxide gas has proven to be a successful sanitizer in a variety of different areas of application. It has already been used to sanitize medical and food processing equipment. It has also been proven to be effective on many fruits and vegetables through a number of research studies. Although it is not approved for direct contact with food materials during processing at this time, there have been a number of studies reporting hopeful results when exposing leafy greens to ClO_2 gas. Of the leafy greens typically seen in the literature, iceberg lettuce is the most common leafy green used in these studies, with much less published work on spinach. A state diagram, based on the 31 data points of color measurement of spinach leaves treated by various combinations of ClO_2 concentration and exposure time, was constructed to describe spinach leaves with no damage, minimal damage and substantial damage based on the color. Then, microbial studies were conducted on the spinach leaves under the various combinations of ClO_2 concentration and exposure time to produce spinach leaves with no damage and minimal damage. The results show that at a ClO_2 concentration of 2mg/l and an exposure time of 20 min, a 2.27 log units bacterial reduction can be achieved with only minor damage to the color of spinach leaves. No damage was observed as a result of the exposure to 4.73mg/l ClO_2 gas for 6 min, which yielded a 2.04 log units bacterial reduction.

From the kinetic data collected for the change in hue angle and microbial reduction, the rate constants for color change and bacterial reduction were determined. The kinetics equations can be utilized to optimize the ClO_2 concentration and exposure time combinations to obtain a minimum change in color of the spinach leaves and a maximum bacterial reduction.

Although more microbial studies must be done to bring further validation to the regions explored in this study, a reduction of 2.27 log units with minimal color damage would suggest ClO_2 has the potential to be used for sanitizing leafy green vegetables. This study also showed a decreasing rate of microbial reduction as exposure time lengthened. For this reason, a high ClO_2 concentration treatment may allow exposure times short enough to avoid damaging the produce completely. Trinetta et al., 2010 has found that the use of high-concentration-short-time processing has had favorable results in the processing of tomatoes. Since ClO_2 residuals have been shown to be low (Han et al., 2004), and the shelf life data suggests ClO_2 has insignificant negative post-treatment effects, high-concentration-short-time show promise for produce sanitation. While further work is needed to understand all of the complex interactions between ClO_2 gas and leafy green vegetables, this work has provided the necessary groundwork to more efficient testing of these interactions.

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Chapter 3: Future Work

The next steps required to advance this research are to increase the quantity of microbial reduction data. In the same way four different concentrations were done for the hue angle change, there should be at least four separate concentrations in order to create a graph of the log of the rate constant against concentration similar to the Figure 2.17. This would provide the change in rate constant as concentration varies so that the hue and microbial reductions could be optimized simultaneously. Once a large body of data had been collected, the effectiveness of ClO_2 on leafy greens could be confirmed.

Further experimentation could be developed to test the effectiveness in a vacuum cooling environment as well as with larger masses of spinach. Approximately 27.8±3.1 g of leaves were exposed in each of the microbial reduction studies. Larger masses could help to demonstrate the penetrability of gaseous sanitizers rather than liquid sanitizers which can be made to look more effective than they are when small samples are used. Both liquid and gaseous sanitizers could be used in hurdle studies. By coupling various liquid sanitizers with various gaseous sanitizers the combined effect of the treatment could be determined. This would work to prove the existence of a synergistic effect of two sanitizers working in combination with one another. It is hypothesized that by combining two sanitizers the resulting log reduction will be larger than the addition of both sanitizers used separately.

Shelf life should also be tested where both the controls and treated samples are inoculated with additional bacteria. Bacteria and fungi could cause the samples to decompose quicker and the positive affect of the ClO_2 treatment could be validated.

The extra washing step in the procedure used in this study should also be tested. Experimentation should be done to see if washing freshly inoculated spinach washes away bacteria that would be more easily killed by chlorine dioxide gas due to biofilms or surface attachment characteristics. If the bacteria being used produces biofilm slowly, or attaches to the sample poorly, it may be easily washed off in the second washing step in this study. This will not only result in a lower initial attachment, but also a reduced log reduction because the most vulnerable bacteria have been washed away.

Experimentation to determine the change in chlorophyll concentration of spinach in relevance to color degradation would also be necessary. Other quality parameters including water activity of spinach leaves and sensory attributes such as taste and nutritional values would have to be considered as well. Quantities of ClO_2 residuals or subsequent byproducts would have to be proven, by FDA standards, to not be a risk to humans.

Finally, if a larger resolution was needed, or a higher concentration needed to be measured, changes could be made to the ClO_2 measurement device to accommodate these requirements. Figure 3.1 shows what voltages would be read if the intensity of UV light shining on the photodiode could be increased. If the open voltage could be at a maximum of V=2.424 at a length of 4 and 6 inches, then the slope and resolution can be found over the desired concentration range. This could be achieved by doing one of two

things. The resistor regulating the amount the amplifier increases the voltage could be changed according to equation P where R_a is the resistor in series with the second amplifier, and R_0 is the resistor in series with the first amplifier (equation 3.1).

$$V_f = V_0 * \frac{R_a}{R_0} = 0.7V * \frac{340K\Omega}{100K\Omega} = 2.38V \quad (3.1)$$

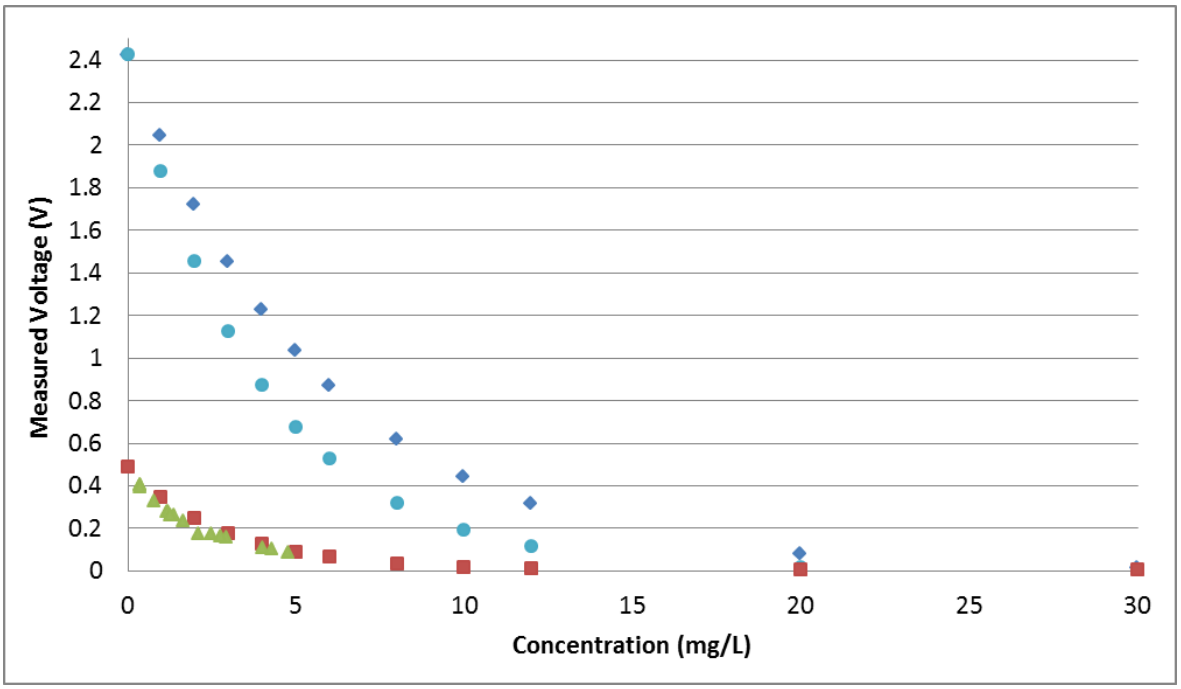


Figure 3.1. Projected increased resolution and concentration measurement capabilities achievable by altering the photodiode measurement device. With the initial voltage of 2.42V and a 4in path length (blue diamonds), V_0 = 2.42V and a 6 in path length (blue circles), V_0 = 0.489V and a path length of 8.2 in (red square), actual V_0 = 0.489V and 8.2 in path length (green triangles).

The second thing that could be done is to increase the intensity of the LED by installing an LED with a larger maximum intensity or shortening the path length.

Appendices

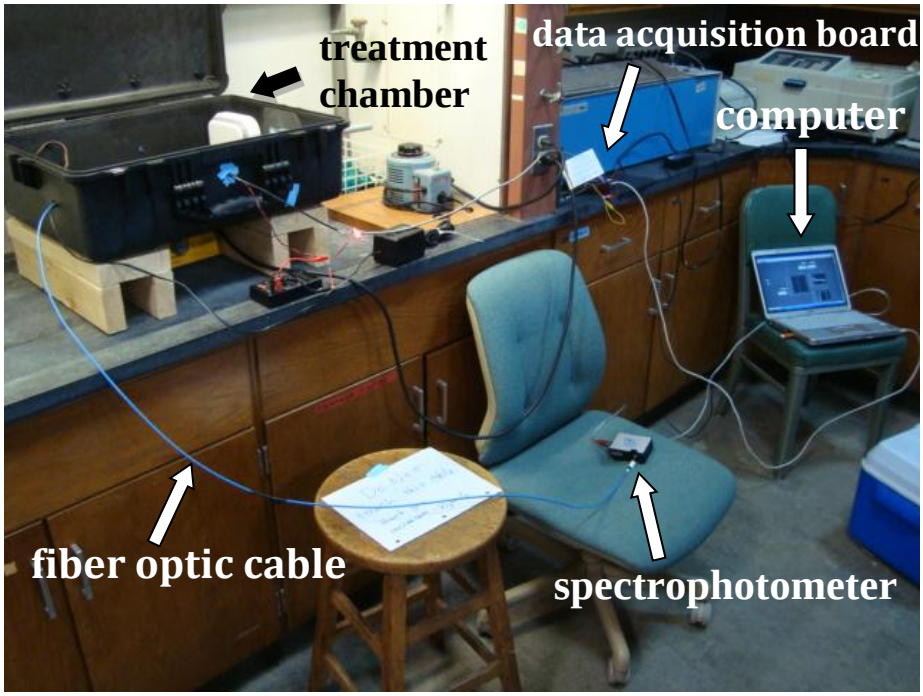
Appendix A: Specifications of Analog Inputs of LabJack U3 Data Acquisition Board

Specifications of Analog Inputs of a LabJack U3 data acquisition board.

Parameter	**Conditions**	**Min**	**Typical**	**Max**	**Units**
Typical Input Range[1]	Single-Ended	0		2.44	volts
	Differential	-2.44		2.44	volts
Max AIN Voltage GND[2]	Valid Operation	-0.3		3.6	volts
Source Impedance[3]				10	kΩ
Resolution (No missing codes)			12		bits
Temperature Drift			15		ppm/°C

(1) With digital to analog convertor (DAC1) disabled, (2) this is the maximum voltage on any analog-in (AIN) pin compared to the ground for valid measurements. Note that a differential channel has a minimum voltage of -2.44 V, meaning that the positive channel can be 2.44 V less than the negative channel, but no AIN pin can go more than 0.3 V below ground, (3) to meet specifications, the impedance of the source signal should be kept at or below the specified value.

Appendix B: Entire System Used in Study



The entire system used in this study.

Appendix C: Calculations to Determine Reactant Quantities

Calculation to determine the quantities of 35% $NaClO_2$ and 6N HCl needed to produce 1 mg/l ClO_2 in the treatment chamber.

$$5NaClO_2 + 4HCl \rightarrow 5NaCl + 4ClO_2 + 2H_2O \quad (1.5)$$

The moles of ClO_2 needed to produce 1 mg/l in the treatment chamber of a volume 44.85L calculated in Equation (C.1).

$$\frac{1mg}{L} * \frac{1g}{1000mg} * \frac{mol}{67.45g} * 44.85L = 6.650x10^{-4} mol\ ClO_2 \quad (C.1)$$

Using the stoichiometric equation we the moles required for each of the reactants can be determined (Equation C.2).

$$8.312x10^{-4}\ NaClO_2 + 6.650x10^{-4}\ HCl \rightarrow 8.312x10^{-4}\ NaCl + 6.650x10^{-4}\ ClO_2 + 3.325x10^{-4}\ H_2O \quad (C.2)$$

Solve the amount of 35% $NaClO_2$ needed using Equation C.3.

$$8.312x10^{-4}\ mol\ NaClO_2 * \frac{90.45g}{1mol} * \frac{1L}{350g} = 0.215mL\ 35\%\ NaClO_2 \quad (C.3)$$

Recalling the $NaClO_2$ is added in 33% excess the final amount required to produce 1 mg/l in the treatment chamber is 0.286 mL.

Solve the amount of 6N HCl needed using Equation C.4.

$$6.650x10^{-4}\ mol\ HCl * \frac{1L}{6mol} = 0.111mL\ 6N \quad (C.4)$$

SPECIFICATIONS

Absolute Maximum Rating (Ta = 25°C)

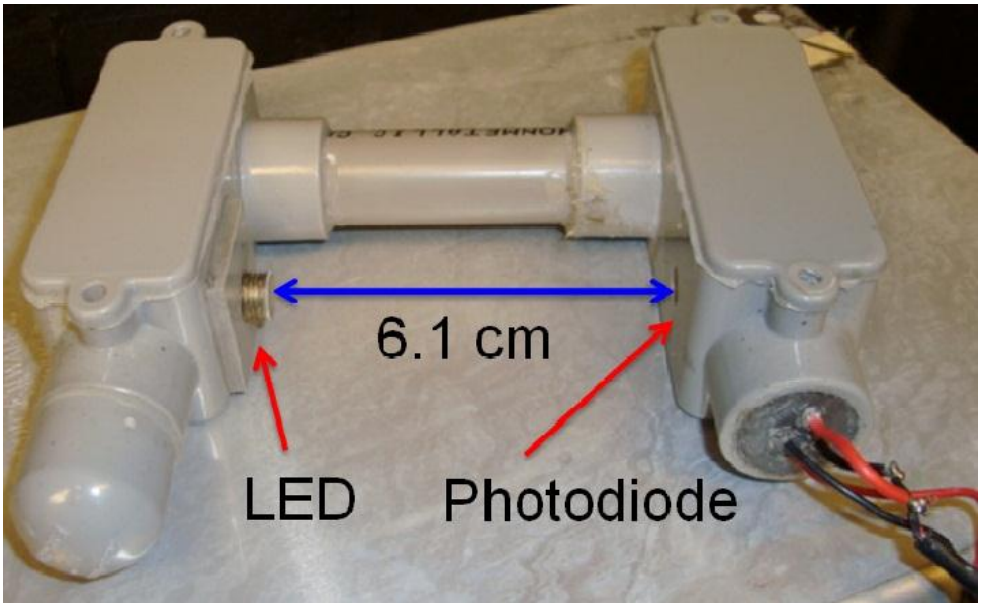
Item	Symbol	Maximum Rating	Unit
DC Forward Current	I_f	30	mA
Pulse Forward Current*	I_{fp}	80	mA
Reverse Current	I_{Rz}	200	mA
Operating Temperature	T_{opr}	-20 to +80	°C
Storage Temperature	T_{stg}	-40 to +100	°C

*Condition: Duty Cycle: 1/10, Pulse Width: 10msec

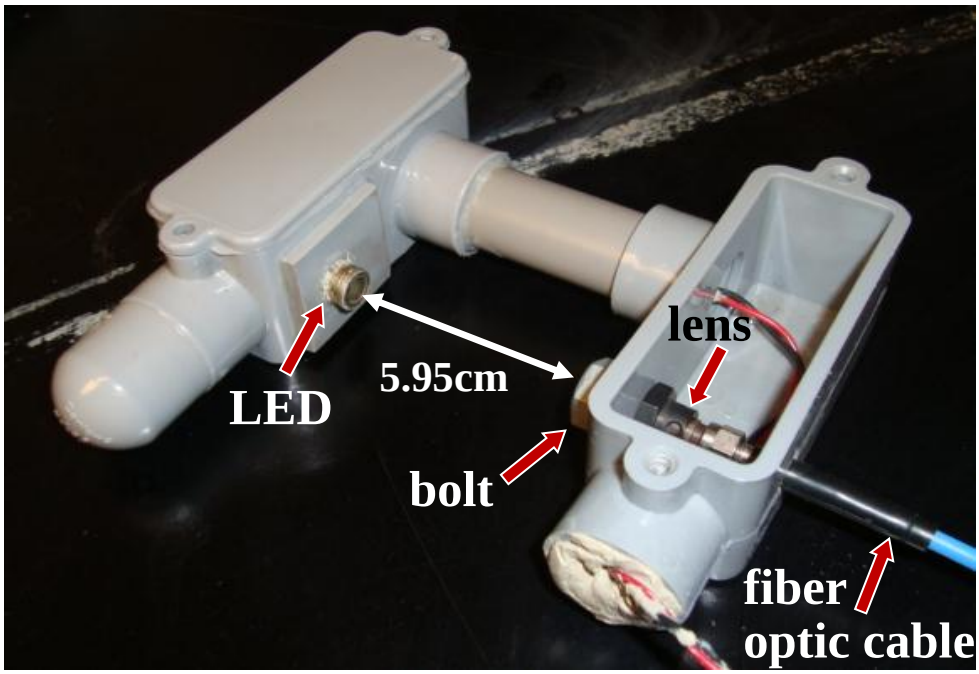
Optical and Electrical Characteristics (Ta = 25°C)

Item	Symbol	Condition	Min	Typ.	Max	unit
Forward Voltage	V_f	I_f=20mA	3.6	4.3	5.0	V
Reverse Current	I_R	V_f =5 V	-	-	100	μA
Peak Wavelength	λ_p	I_f=20mA	350	352	355	nm
Viewing Angle		I_f=20mA	-	10	-	deg.
Output Power/Flux	P_o	I_f=20mA	50	100	200	μW

Appendix E: Measurement Unit Photographs

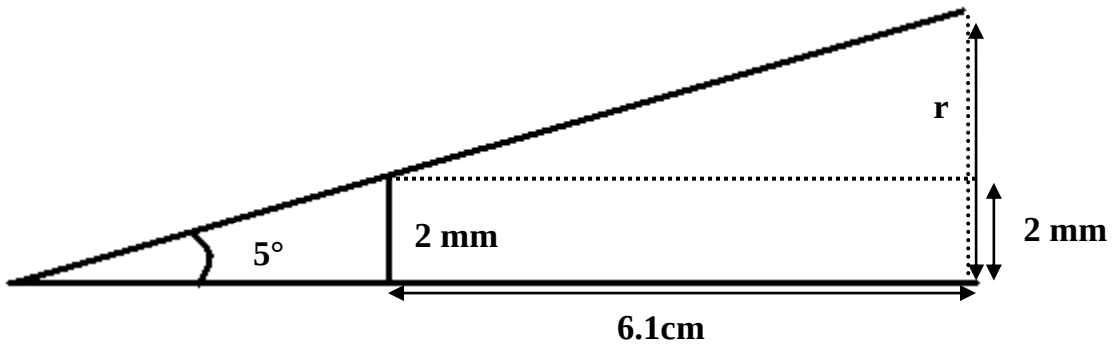


Photodiode measurement unit.



Spectroscopic measurement unit.

Appendix F: Calculations to Determine the Resistors to be used Around the Amplifier.



A distance of 6.1cm from the LED to the photodiode in order to calculate how much the UV light from the LED spreads.

Using the tangent of the angle given we can determine the radius of the projected UV light.

$$\tan 5° = \frac{opp}{6.1\ cm} \rightarrow opp = 0.534cm$$

$$r = 0.534cm + 2mm = 7.34mm$$

$$A = \pi r^2 = \pi * 7.34^2 = 169mm^2$$

Since the area of the active region on the photodiode was 4.84 mm^2, 2.9% of the beam was actually striking the active region. The amount of power that was actually utilized can be calculated;

$$700\mu W * 0.029 = 20.3\mu W$$

The photocurrent corresponding to the calculated power is then determined.

$$I_P = 0.1\ {}^{A}/_{W} * 2.03x10^{-5}W = 0.00203mA$$

$$R = \frac{V}{I} = \frac{2.44V}{2.03x10^{-6}} = 1{,}201{,}970\Omega$$

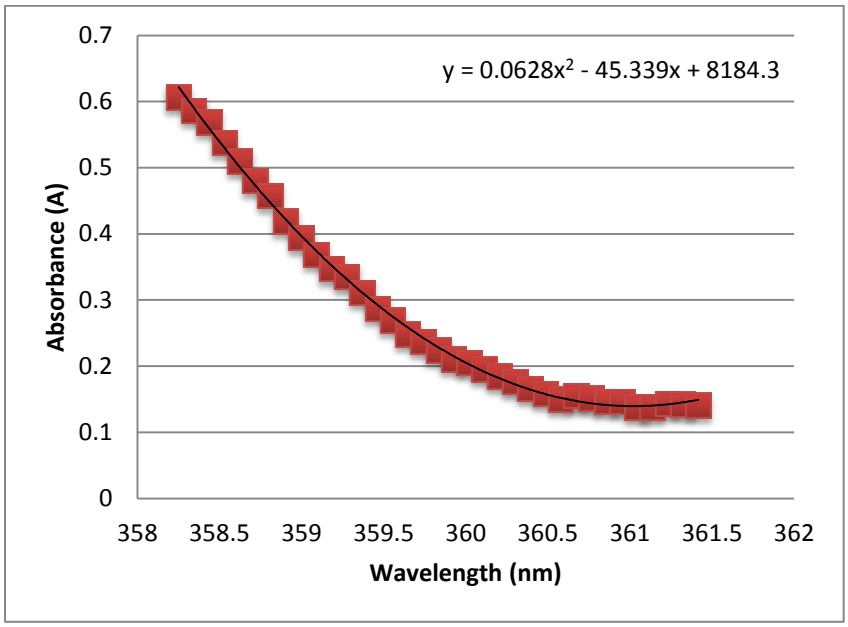
If a resistor of about 1.2 MΩ was used then it is expected to have an initial voltage of 2.44V, the maximum voltage the data acquisition board is capable of recording. Using a 100kΩ resistor, initial and final voltages of approximately 700mV and 100mV, respectively, were found. Calculating what the actual power was using this voltage and 100 KΩ resistors the power is found to be:

$$I = \frac{V}{R} = \frac{0.7V}{100{,}000\Omega} = 0.007mA$$

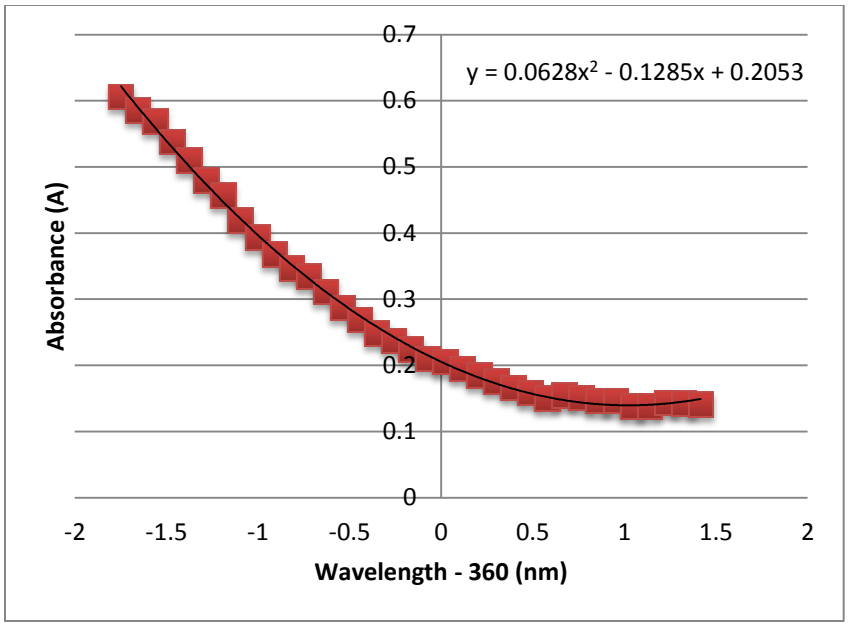
This value is much larger than the 0.00203 mA calculated from the percent of the beam striking the active region of the photodiode.

Appendix G: Equation of the Spectrophotometer Polynomial

Example of an equation of the polynomial fit to pixels 358.45-361.05 nm of the Ocean Optics USB4000 spectrophotometer to determine absorbance at exactly 360 nm.



To avoid rounding errors, subtract each wavelength by 360 before plotting the data and determining the equation of the polynomial.



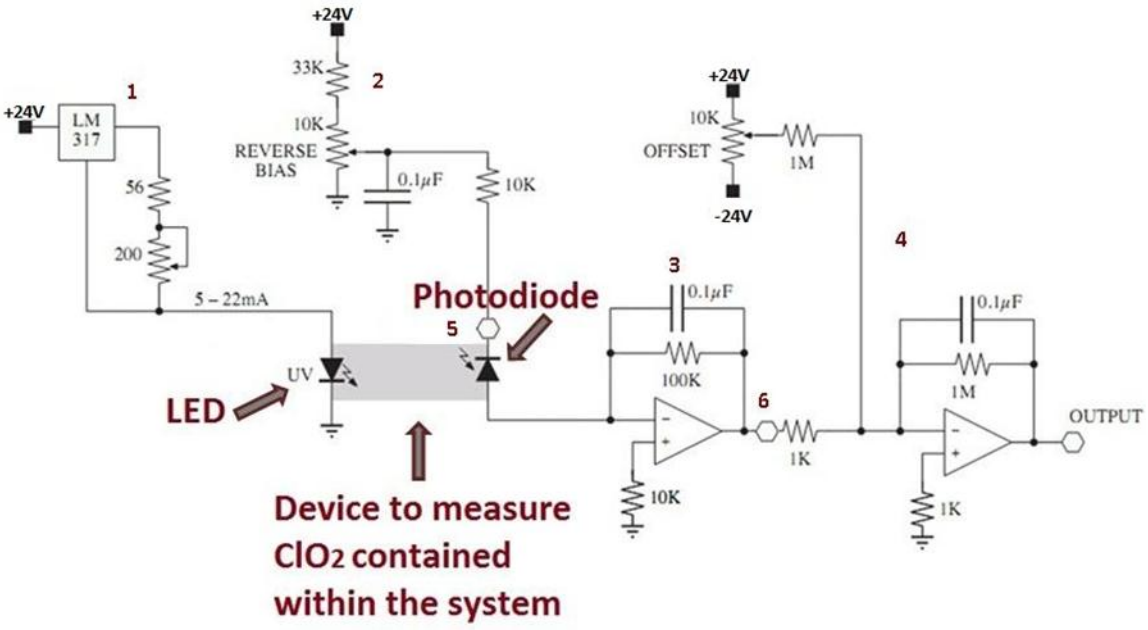
So the exact absorbance at 360 nm is the intercept of the equation

$$y = 0.0628x^2 - 0.1285x + 0.2053$$

$$A(360) = 0.2053A$$

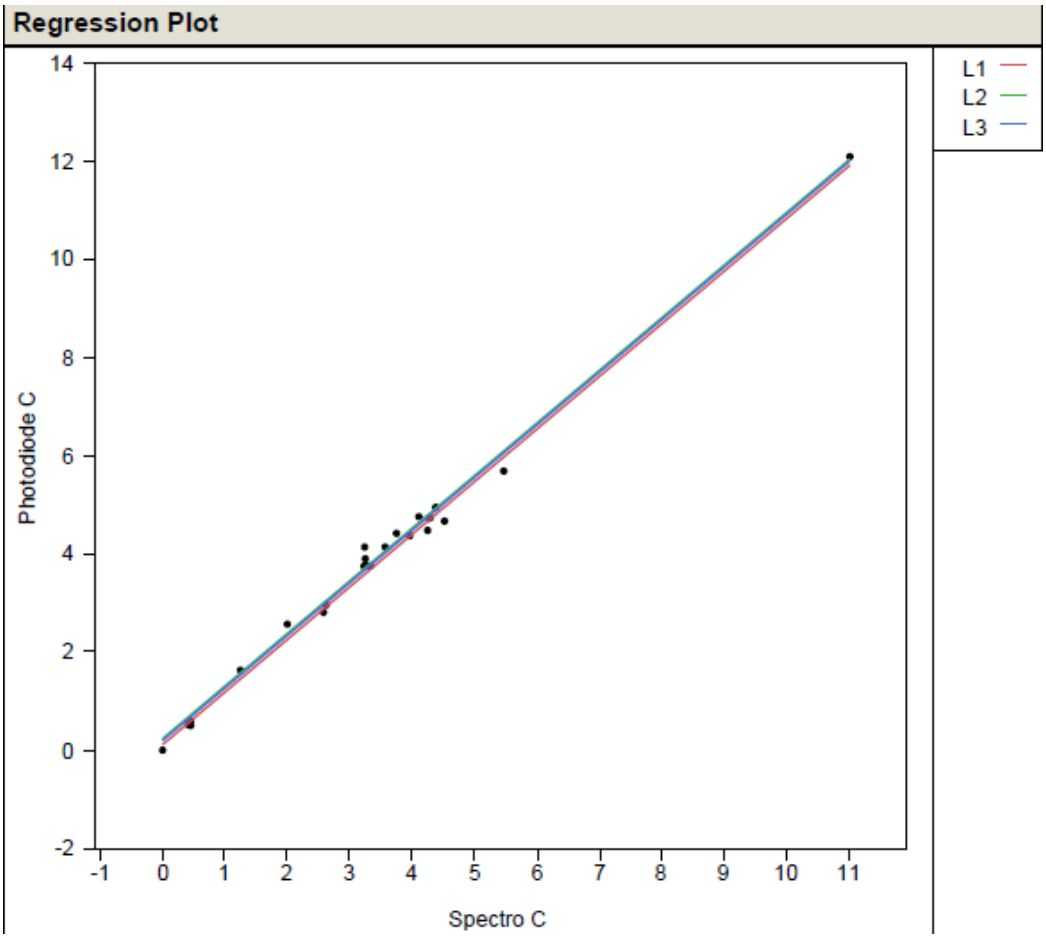
Appendix H: Original Circuit Design

Preliminary circuit design of the voltage measurement unit discussed in section 1.5.1. Two amplification components are shown (component 3 and 4) over amplifying the voltage signal being read at the output. The final circuit design only utilized amplification component 3.



Appendix I: Statistical Analysis Report Comparing Old and New Measurement Device

Select statistical analysis reports where L1 is the concentration data collected using the old embodiment, L2 is collected using the new embodiment, and L3 is a compilation of both the old and new embodiment.



LSMeans Differences Tukey HSD

Level		Least Sq Mean
L2	A	3.5275863
L3	A	3.4859259
L1	A	3.4026051

Levels not connected by same letter are significantly different.

Appendix J: Resistor Consideration to Prevent Circuit Current Drift

To determine if the difference in resistance could account for the increase in current, the two resistors in parallel (56Ω and 180Ω) were considered (equation G.1).

$$\frac{1}{R_T} = \frac{1}{56\Omega} + \frac{1}{180\Omega} \rightarrow R_T = 42.7\Omega \qquad (G.1)$$

The average initial current when the LEDs were turned on was I_i = 29.5 mA, so the voltage is calculated using equation G.2.

$$V = IR = 29.5mA * 42.7\Omega = 1.26V \qquad (G.2)$$

When using a 1,000,000Ω the resistance through the resistors could change a maximum of -450Ω/°C. Since we are using only 42.7Ω, the maximum change in resistance is calculated (equation G.3).

$$\frac{-450\Omega/°C}{1{,}000{,}000\Omega} = \frac{x}{42.7\Omega} \rightarrow x = -4.5x10^{-4}/°C * 42.7\Omega = -0.0192\,\frac{\Omega}{°C} \qquad (G.3)$$

Using the final current (equation G.4), a final resistance is calculated (G.5). The final resistance is the value that the resistance would have to be in order for the resistors to be the singular cause of the current drift observed in the circuit.

$$I_i = 29.5mA, I_f = 29.5mA + 1.5mA = 31.0mA \qquad (G.4)$$

$$R_f = \frac{V}{I_f} = \frac{1.26V}{31.0mA} = 40.6\Omega \qquad (G.5)$$

The difference between the final and initial resistance (equation G.6) is -2.1Ω, so the total temperature change required to cause this change in resistance is calculated (equation G.7).

$$\Delta\Omega = 40.6\Omega - 42.7\Omega = -2.1\Omega \qquad (G.6)$$

$$\frac{-2.1\Omega}{-0.0192\,{}^{\Omega}/_{\circ C}} = 109°C\ increase \qquad (G.7)$$

Appendix K: Relevant Data Acquisition Board Calculations

The data acquisition board has a 12-bit resolution and a maximum voltage of 2.44 V, so the smallest increment measureable is calculated in equation H.1.

$$\frac{1}{2^{12}} * 2.44V = 0.595mV \qquad (H.1)$$

The spectrophotometer has 16-bit resolution and a maximum intensity of 32,000 counts so the maximum absorbance resolution is calculated using equation H.2.

$$A_A = -log\frac{I_m - I_d}{I_o - I_d} * \frac{1}{2^{16}} = -log\frac{1-0}{32{,}000-0} * \frac{1}{2^{16}} = 6.88x10^{-5}A \qquad (H.2)$$

where A_A is the absorbance from the spectroscopic measurement unit. The voltage values must be converted to an absorbance value to compare between the data acquisition board and the spectrophotometer (equation H.3 and H.4). The initial voltage was taken to be V_0 = 687mV, the average initial value of the voltage reading inside the treatment chamber.

$$A_P = -log\frac{V_m - V_d}{V_o - V_d} = -log\frac{687mV - 0.595mV - 3mV}{687mV - 3mV} = 3.78x10^{-4}A \qquad (H.3)$$

where A_P is the absorbance calculated from the photodiode measurement unit. Since $3.78x10^{-4}$A is larger than $6.88x10^{-5}$ A, the data acquisition board is considered to be the component limiting the resolution of the system. Looking now at three points from Figure 1.11 the concentration was calculated for each point using equations 1.1 and 1.2.

$$Approximately\ 2\frac{mg}{L}: A = -log\frac{407mV - 3mV}{684mV - 3mV} = 0.230A$$

$$c = \frac{A}{l\varepsilon} = \frac{0.230A}{6.1cm * 1250M^{-1}cm^{-1}} = 2.04\frac{mg}{L}$$

$$Approximately\ 5\frac{mg}{L}: A = -log\frac{191mV - 3mV}{679mV - 3mV} = 0.560A$$

$$c = \frac{A}{l\varepsilon} = \frac{0.560A}{6.1cm * 1250M^{-1}cm^{-1}} = 4.95\frac{mg}{L}$$

$$Approximately\ 12\frac{mg}{L}: A = -log\frac{33mV - 3mV}{697mV - 3mV} = 1.368A$$

$$c = \frac{A}{l\varepsilon} = \frac{1.368A}{6.1cm * 1250M^{-1}cm^{-1}} = 12.10\frac{mg}{L}$$

The affect 0.595mV has at these three concentrations were determined using equations 1.1 and 1.2.

$$Approximately\ 2\frac{mg}{L}: A = -log\frac{407.595mV - 3mV}{684mV - 3mV} = 0.226A$$

$$c = \frac{A}{l\varepsilon} = \frac{0.226A}{6.1cm * 1250M^{-1}cm^{-1}} = 2.00\frac{mg}{L}$$

$$Resolution\ at\ approximately\ 2\frac{mg}{L}: 2.04\frac{mg}{L} - 2.00\frac{mg}{L} = 0.04\frac{mg}{L}$$

$$Approximately\ 5\frac{mg}{L}: A = -log\frac{191.595mV - 3mV}{679mV - 3mV} = 0.554A$$

$$c = \frac{A}{l\varepsilon} = \frac{0.554A}{6.1cm * 1250M^{-1}cm^{-1}} = 4.90\frac{mg}{L}$$

$$Resolution\ at\ approximately\ 5\frac{mg}{L}: 4.95\frac{mg}{L} - 4.90\frac{mg}{L} = 0.05\frac{mg}{L}$$

$$Approximately\ 12\frac{mg}{L}: A = -log\frac{33.595mV - 3mV}{697mV - 3mV} = 1.356A$$

$$c = \frac{A}{l\varepsilon} = \frac{1.356A}{6.1cm * 1250M^{-1}cm^{-1}} = 12.00\frac{mg}{L}$$

$$Resolution\ at\ approximately\ 12\frac{mg}{L}: 12.10\frac{mg}{L} - 12.00\frac{mg}{L} = 0.10\frac{mg}{L}$$

Conservatively the resolution of the ClO_2 concentration measurement system is 0.04 mg/l from 0-2 mg/l and 0.05 mg/l from 2-5 mg/l.

Appendix L: Full Data used to Comprise State Diagram

Evaluation of no damage (green), minimal damage (yellow), and substantial damage (red) of all 31 samples used to develop figure 2.16.

Conc.	Time	Ho average	Hue final worst	ΔHw	Hw/Ho
1.99	6	120.37	120.09	-0.27	0.997732
2.06	12	119.32	119.39	0.07	1.000626
2.04	24	116.87	116.46	-0.41	0.99648
2.07	3	120.13	120.43	0.30	1.002495
5.96	2	117.55	110.56	-6.99	0.940535
6.02	1	118.61	117.49	-1.11	0.990603
5.78	0.5	118.89	118.15	-0.74	0.993765
5.76	3	117.08	113.92	-3.16	0.973031
3.75	2	117.61	117.53	-0.09	0.999268
3.76	8	117.75	116.80	-0.95	0.991915
3.75	16	117.85	112.90	-4.95	0.958027
3.75	24	117.90	113.08	-4.82	0.959133
4.14	10	118.33	118.02	-0.30	0.997427
4.42	3.5	117.79	116.97	-0.83	0.992991
3.90	15	117.43	116.40	-1.03	0.991205
4.95	3	118.06	117.26	-0.80	0.99321
4.73	6	117.13	114.21	-2.92	0.975101
4.74	10	118.23	115.64	-2.59	0.978063
4.76	4	116.95	115.46	-1.49	0.987267
4.87	14	117.62	114.39	-3.23	0.972521
5.69	2	117.41	116.62	-0.79	0.993241
2.57	24	117.65	117.29	-0.36	0.996945
4.14	15	119.59	118.65	-0.95	0.992096
2.56	12	120.85	119.62	-1.22	0.989864
2.00	3	120.35	119.56	-0.79	0.993418
2.16	20	117.53	116.25	-1.28	0.989082
4.60	6	119.19	118.35	-0.84	0.992967
5.95	2	120.29	118.92	-1.36	0.988671
4.60	10	118.96	117.42	-1.53	0.987117
4.68	14	118.69	115.30	-3.39	0.971408
4.70	0.5	117.21	116.51	-0.71	0.993963

Appendix M: Entire Plate Count Data Used in Microbiological Studies

Raw data from microbial reduction experiments used in this study.

Sample #8	Discription	10^0	10^-1	10^-2	10^-3	10^-4	10^-5	10^-6	Concentration (cells/ml)	Average	LOG RED	±
1.8	IT	TNTC	81.0	6.0					8.10E+03			
2.8	IT	TNTC	142.0	23.5					1.42E+04			
3.8	IT	TNTC	72.5	5.0					7.25E+03	4.08		± 0.27
4.8	IT	TNTC	105.5	3.0					1.06E+04			
5.8	IT	TNTC	228.0	16.5					2.28E+04			
6.8	IT	TNTC	95.5	12.5					9.55E+03		**1.28**	
7.8	I/NT		TNTC	92.5	5.0	0.0			9.25E+04			
8.8	I/NT		TNTC	TNTC	35.0	1.5			3.50E+05	5.36		± 0.39
9.8	I/NT		TNTC	311.5	24.0	2.0			2.40E+05			
10.8	I/NT		TNTC	221.0	16.0	1.5			2.21E+05			
11.8	NI/NT		0.3	0.0					n/a			
inoc	inocula				TNTC	317.0	31.0	1.0	3.17E+07	7.50		± 0.06
Sample #9	**Discription**	**10^0**	**10^-1**	**10^-2**	**10^-3**	**10^-4**	**10^-5**	**10^-6**	**Concentration (cells/ml)**	**Average**	**LOG RED**	**±**
1.9	I/T	376.0	6.0	2.5					3.76E+03			
2.9	I/T	TNTC	25.5	4.0					2.55E+03	3.53		± 0.12
3.9	I/T	381.0	13.0	1.5					3.81E+03			
4.9	I/T	452.0	23.5	3.5					3.44E+03			
5.9	NI/T								for color			
6.9	NI/T								for color		**1.20**	
7.9	I/NT			28.0	2.0	2.0			2.80E+04			
8.9	I/NT			84.5	6.5	1.5			8.45E+04	4.73		± 0.29
9.9	I/NT			64.5	5.0	0.0			6.45E+04			
10.9	I/NT			36.0	3.5	0.5			3.60E+04			
12.9	NI/NT								for color			
inoc	inocula					111.0	8.0	0.0	1.11E+07	7.05		± 0.23
Sample #10	**Discription**	**10^0**	**10^-1**	**10^-2**	**10^-3**	**10^-4**	**10^-5**	**10^-6**	**Concentration (cells/ml)**	**Average**	**LOG RED**	**±**
1.10	I/T	176.0	5.5	1.0	0.0				1.76E+03			
2.10	I/T	73.0	2.0	0.0	0.0				7.30E+02	3.24		± 0.18
3.10	I/T	269.0	10.5	4.0	0.0				2.69E+03			
4.10	I/T	178.0	9.5	1.0	0.0				1.78E+03			
5.10	NI/T								for color			
6.10	NI/T								for color		**2.27**	
7.10	I/NT			98.0	8.5	1.0			9.80E+04			
8.10	I/NT			TNTC	64.0	7.0			6.40E+05	5.52		± 0.53
9.10	I/NT			TNTC	24.5	2.5			2.45E+05			
inoc	inocula					TNTC	80.5	15.5	8.05E+07	7.91		± 0.23
Sample #11	**Discription**	**10^0**	**10^-1**	**10^-2**	**10^-3**	**10^-4**	**10^-5**	**10^-6**	**Concentration (cells/ml)**	**Average**	**LOG RED**	**±**
1.11	I/T	285.0	17.0	1.0					2.85E+03			
2.11	I/T	178.0	13.5	0.0					1.78E+03	3.37		± 0.17
3.11	I/T	308.0	14.5	3.0					3.08E+03			
4.11	I/T	160.0	11.0	1.0					1.60E+03			
5.11	NI/T								for color			
6.11	NI/T								for color		**2.04**	
7.11	I/NT			134.0	15.0	0.5			1.34E+05			
8.11	I/NT			TNTC	76.0	2.0			7.60E+05	5.41		± 0.50
9.11	I/NT			49.5	5.0	0.5			4.95E+04			
10.11	I/NT			82.0	1.5	2.0			8.20E+04			
inoc	inocula					TNTC	TNTC	51.0	5.10E+08	8.71		± 0.03

Sample # 12	Discription	10^0	10^-1	10^-2	10^-3	10^-4	10^-5	10^-6	Concentration (cells/ml)	Average	LOG RED	±
1.12	I/T	TNTC	TNTC	33.0					3.30E+04			
2.12	I/T	TNTC	TNTC	75.0					7.50E+04	4.67		± 0.31
3.12	I/T	TNTC	TNTC	57.0					5.70E+04			
4.12	I/T	TNTC	111.0	34.5					2.28E+04			
5.12	NI/T								for color			
6.12	NI/T								for color		**0.79**	
7.12	I/NT			TNTC	50.0	7.5			5.00E+05			
8.12	I/NT			TNTC	40.0	16.0			4.00E+05	5.46		± 0.40
9.12	I/NT			114.5	18.5	1.5			1.15E+05			
10.12	I/NT			141.0	9.0	2.0			1.41E+05			
inoc	inocula					TNTC	TNTC	24.0	2.40E+08	8.38		± 0.00
Sample # 13	**Discription**	**10^0**	**10^-1**	**10^-2**	**10^-3**	**10^-4**	**10^-5**	**10^-6**	**Concentration (cells/ml)**	**Average**	**LOG RED**	**±**
1.13	I/T	507.0	30.0	5.0					4.04E+03			
2.13	I/T	596.0	37.5	6.5					3.75E+03	3.57		± 0.12
3.13	I/T	478.0	38.5	8.0					4.32E+03			
4.13	I/T	543.0	28.0	6.0					2.80E+03			
5.13	NI/T								for color			
6.13	NI/T								for color		**2.23**	
7.13	I/NT			TNTC	168.0	6.5			1.68E+06			
8.13	I/NT			245.5	15.0	2.5			2.46E+05	5.80		± 0.45
9.13	I/NT			TNTC	39.0	7.5			3.90E+05			
10.13	I/NT			223.5	22.5	3.5			2.24E+05			
inoc	inocula					TNTC	164.0	15.5	1.64E+08	8.21		± 0.03
Sample # 14	**Discription**	**10^0**	**10^-1**	**10^-2**	**10^-3**	**10^-4**	**10^-5**	**10^-6**	**Concentration (cells/ml)**	**Average**	**LOG RED**	**±**
1.14	I/T	35.0	2.0	0.0					3.50E+02			
2.14	I/T	1.0	0.0	0.0					1.00E+01	2.46		± 0.06
3.14	I/T	28.0	1.0	0.0					2.80E+02			
4.14	I/T	28.0	2.0	0.0					2.80E+02			
5.14	NI/T								for color			
6.14	NI/T								for color		**2.37**	
7.14	I/NT			59.0	43.0	1.5			5.90E+04			
8.14	I/NT			85.5	7.5	0.5			8.55E+04	4.83		± 0.46
9.14	I/NT			29.5	3.0	0.0			2.95E+04			
10.14	I/NT			95.5	21.5	0.0			9.55E+04			
inoc	inocula					TNTC	28.5	2.5	2.85E+07	7.45		± 0.02
Sample # 15	**Discription**	**10^0**	**10^-1**	**10^-2**	**10^-3**	**10^-4**	**10^-5**	**10^-6**	**Concentration (cells/ml)**	**Average**	**LOG RED**	**±**
1.15	I/T	TNTC	45.5	5.0	0.0				4.55E+03			
2.15	I/T	TNTC	132.0	24.0	6.0				1.32E+04	4.05		± 0.39
3.15	I/T	TNTC	133.0	56.0	1.0				1.33E+04			
4.15	I/T	TNTC	137.5	19.5	0.0				1.38E+04			
5.15	NI/T								for color			
6.15	NI/T								for color		**1.18**	
7.15	I/NT			151.5	6.5	4.5			1.52E+05			
8.15	I/NT			56.0	24.5	3.0			5.60E+04	5.23		± 0.52
9.15	I/NT			TNTC	41.0	4.5			4.10E+05			
10.15	I/NT			58.0	4.5	1.5			5.80E+04			
inoc	inocula					TNTC	188.5	14.5	1.89E+08	8.28		± 0.36

Appendix N: Entire Shelf Life Data Used in Hue Analysis

Raw shelf life data used to create table 2.6.

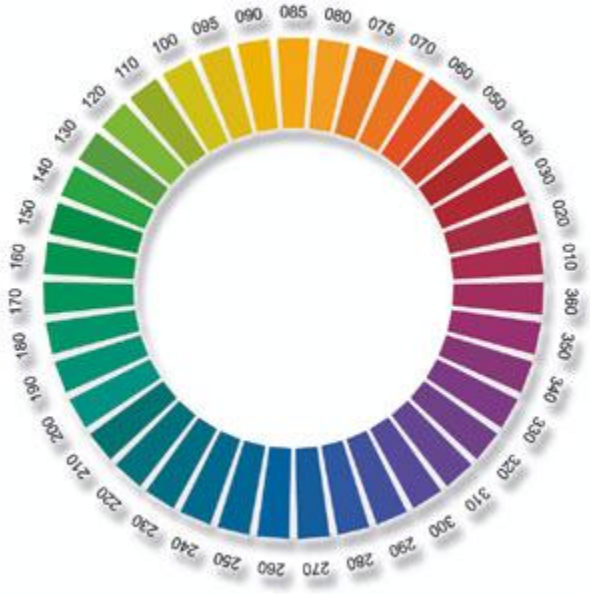
Experiment	2		4		5		6	
Concentration	4.60 mg/L		4.60 mg/L		4.68 mg/L		4.65 mg/L	
Exposure Time	6 min		10 min		14 min		0.5 min	
Samples	Control	5 & 6 average	Control	5 & 6 average	Control	5 & 6 average	Control	5 & 6 average
Initial Hue	120.01	119.19	118.88	118.96	118.98	118.69	117.71	117.21
0 day	120.64	118.35	117.90	117.42	118.28	115.30	116.74	116.51
7 day	118.84	116.21	116.55	115.04	116.39	112.64	114.99	109.73
14 day	117.48	113.34	115.75	111.78	113.99	110.90	112.92	102.83

Appendix O: Additional Photographs of Upper and Lower Spinach Leaves

The figure below shows both before (upper) and after (bottom) treatment with 3.95 mg/l ClO_2 gas for 15.5 minutes For the upper leaves the average H_0 = 120.3, H_w =118.9, and H_w/H_0 = 0.988.

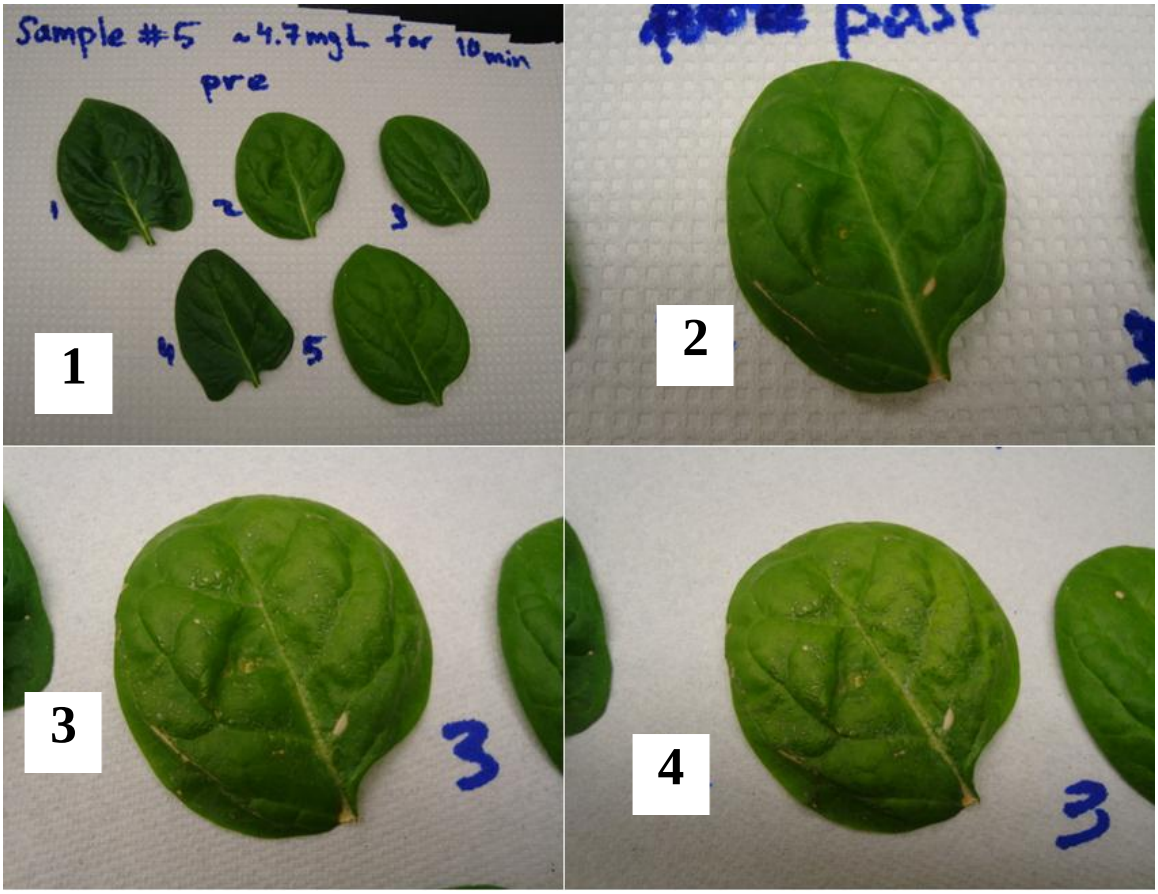
Upper leaves (left) and lower leaves (right), both before (top) and after (bottom) treatment with 3.95 mg/l ClO_2 gas for 15.5 minutes.

For the lower leaves the average H_0 = 113.4, H_w =107.3, and H_w/H_0 = 0.946. The damage sustained by the lower leaves was clearly visible, where there was no visible damage in the upper leaves as seen in the figure. The figure below shows what each hue angle looks like at a standard lightness.



The CIELAB color wheel showing the base color at different hue angles (Konica Minolta Sensing, Inc., 2007).

Appendix P: Various Photograph from Shelf Life Study



Sample treated with 4.60 mg/l for 10 minutes shown at 0 days (1), immediately after exposure (2), 7 days (3), and 14 days (4).

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